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ATTENUATING ORGANIC CONTAMINANT MOBILITY BY SOIL MODIFICATION: TOWARDS A BIOLOGICALLY INTEGRATED TECHNOLOGY

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Coupling the enhanced retention of nonionic organic contaminants (NOCs) in quaternary ammonium (QUAT)-amended subsoils with bioremediation of the immobilized NOCs is being studied as a comprehensive soil restoration technology. Four areas were investigated: 1) QUAT binding to soils and subsoils, 2) QUAT biocompatibility with NOC-degrading microorganisms, 3) biostability of QUATs exchanged onto natural soils and clays, and 4) NOC bioavailability to indigenous microorganisms. HDTMA-clay complexes are chemically stable when hydrophobic HDTMA bonding is limited by lowering the ionic strength and controlling companion ions. HDTMA biostability can be increased by: 1) binding to clay exchange sites, 2) introduction to subsoils rather than surface soils and 3) maintaining saturated soil conditions. Although HDTMA is toxic to axenic cultures of bacteria, its toxicity is virtually eliminated by binding to clay minerals. Repopulation of the treated zone should occur once HDTMA is bound to soil. Contaminants sorbed to HDTMA-soils should be bioavailable to bacteria since NOC desorption rates from these materials are fast and some degradative bacteria have the ability to directly utilize sorbed contaminants.

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PREFACE

This report was prepared by Michigan State University, Department of Crop and Soil Sciences, Plant and Soil Sciences Building, East Lansing, MI 48824-1325, USAF Contract No. F08635-91C-0173, for the Armstrong Laboratory Environics Directorate (AL/EQ), Suite 2, 139 Barnes Drive, Tyndall Air Force Base, Florida 32403-5319.

This final report describes a comprehensive *in situ* technology that combines non-ionic contaminant (NOC) immobilization with *in situ* biodegradation of the NOCs as an integrated approach for the remediation of contaminated aquifer systems. It details the cation exchange chemistry of hexadecyltrimethyl ammonium (HDTMA) in a subsoil, the biostability of HDTMA in soil and clay systems, the biocompatability of HDTMA to soil microorganisms and the bioavailability of naphthalene to microorganisms when sorbed to HDTMA-smectite.

The work was performed between June 1991 and June 1994. The AL/EQW project officer was Capt. Jeffrey Stinson.

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EXECUTIVE SUMMARY

A. OBJECTIVE

The primary objective of this project is to develop a comprehensive *in situ* technology that combines non-ionic contaminant (NOC) immobilization with *in situ* biodegradation of the NOCs as an integrated approach for the remediation of contaminated aquifer systems.

B. BACKGROUND

Soil organic matter (SOM) plays a predominant role in the sorption of NOCs from water. Interactions between NOCs and the SOM resemble a partitioning process in which SOM acts as a bulk organic solvent. The sorptive capacity of low organic matter soils can be increased through the addition of quaternary ammonium compounds (QUATs) which displace exchangeable cations from soil clays resulting in a low polarity organic partition phase. This phase is more effective than natural SOM in removing NOCs from solution. Coupling NOC immobilization with *in situ* biodegradation of the NOCs by indigenous soil or aquifer microorganisms would constitute a comprehensive soil restoration technology.

C. SCOPE

This document presents and discusses our findings on HDTMA exchange chemistry, HDTMA biostability in soils, HDTMA biocompatibility to microorganisms and naphthalene bioavailability from HDTMA-smectite. Section I is an introduction to the proposed aquifer remediation technology. Section II discusses the aspects of HDTMA exchange chemistry in subsoils. Section III details the biocompatibility of HDTMA with soil microorganisms and its impact on NOC degradative capabilities and rates. Section IV discusses the biological stability of HDTMA-clay or -soil complexes in soil systems. Section V examines the availability of naphthalene to microorganisms when associated with HDTMA-smectite complexes. Section VI details the conclusions of this report and recommendations are made in Section VII. Materials and methods employed in this study are described in each section.

D. CONCLUSIONS

Coupling *in situ* immobilization of NOCs with their subsequent biological degradation is an attractive aquifer remediation technology. HDTMA soil complexes are chemically stable as long as the amount of HDTMA adsorption by hydrophobic bonding is limited by lowering ionic strength and controlling the companion ions of HDTMA. HDTMA is generally biologically stable in soils and this stability can be increased by 1) binding to clay exchange sites, especially internal sites (e.g., smectite), 2) introduction to subsoils rather than surface soils and 3) maintaining saturated soil conditions. The treatment of soils and aquifer materials with HDTMA affected the activities of aerobic, heterotrophic bacteria. However, results indicate that a portion of the microbial population should survive HDTMA treatment, thereby retaining some degradative capability and that repopulation of the treated zone should occur once HDTMA is bound to the soil. Contaminants sorbed to HDTMA-modified soils should be bioavailable to bacteria since NOC desorption rates from these materials are fast and some degradative bacteria have the abililty to directly utilize sorbed contaminants.

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LIST OF ACRONYMS

BSM	Basal Salts Medium
CEC	Cation Exchange Capacity
DODMA	Dioctadecyldimethylammonium
DTDMAC	Ditallowdimethylammonium chloride
ETS	Electron Transport System
FDA	Fluroescein diacetate
HDTMA	Hexadecyltrimethylammonium
INT	2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium salt
MIC	Minimum growth Inhibitory Concentration
NOC	Nonionic Organic Contaminant
PBS	Phosphate Buffered Saline solution
ppm	parts per million (mg/L)
PTYG	Peptone Tryptone Yeast Extract Glucose medium
QUAT	Quaternary Ammonium Compound
SOM	Soil Organic Matter
STAC	Stearyltrimethylammonium chloride

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SECTION I

INTRODUCTION

A. OBJECTIVE

The primary objective of this project is to develop a comprehensive *in situ* technology that combines non-ionic contaminant (NOC) immobilization with *in situ* biodegradation of the NOCs as an integrated approach for the remediation of contaminanted aquifer systems.

B. BACKGROUND

Soil organic matter (SOM) plays a predominant role in the sorption of NOCs from water. Interactions between NOCs and the SOM resemble a partitioning process in which SOM acts as a bulk organic solvent (Chiou et al. 1979; Chiou et al. 1983; Karickhoff, 1979). Mineral phases in soils are generally ineffective as sorbents of NOCs due to the polar nature of minerals such as silica and metal oxides and the presence of hydrated exchange ions such as Na^{+}, K^{+}, Mg^{++}, and Ca^{++} on clay surfaces (Chiou and Shoup 1985). Therefore, low organic matter surface soils and subsoils have a limited capability to sorb NOCs, increasing the potential for NOC transport in the soil profile.

Recently, we have developed a soil modification approach for reducing contaminant transport in soils, subsoils and aquifer materials. The sorptive capacity of these soils can be increased

through the addition of quaternary ammonium compounds (QUATs) of the general form $[(CH_3)_3NR]^+$ or $[(CH_3)_2NRR']^+$ where R and R' are alkyl hydrocarbon moieties. Our studies have focused on QUATs with alkyl chains of nine carbon atoms or more including hexadecyltrimethylammonium (HDTMA) or dioctadecyldimethyl-ammonium (DODMA). When added to soil, these cations displace native exchangeable inorganic cations from soil clays resulting in the formation of a low polarity, organic partition phase comprised of the interacting alkyl hydrocarbon tails. Lee et al. (1989) demonstrated that the sorption coefficients (K values) of several aromatic hydrocarbons (e.g., benzene, toluene, and ethylbenzene) could be increased by over two orders of magnitude in B-horizon subsoils treated with HDTMA in an amount equivalent to the cation exchange capacity (CEC) of the soil. Increases in K values of this magnitude substantially decrease the mobility and leaching potential of organic contaminants, such as benzene, which are commonly found in groundwater. The organic sorptive phase formed in the HDTMA-treated soils studied by Lee et al. (1989) was 10 to 30 times more effective on a carbon unit mass basis than natural SOM in removing NOCs from solution. HDTMA forms a low polarity organic phase derived from the interacting tails, whereas SOM contains more polar groups which lower its effectiveness as a partition phase for organic compounds.

Coupling NOC immobilization with *in situ* biodegradation of the NOCs by indigenous soil or aquifer microorganisms would constitute a comprehensive soil remediation technology. *In situ* formation of organo-clay complexes from naturally-occurring clays (i.e., those present in subsoils or aquifer materials) could be accomplished by injection of the QUAT into the subsurface to create a sorptive zone that would retard migration and concentrate target contaminants within a confined area (Boyd et al. 1988, 1991; Burris and Antworth 1992). The feasibility of this concept was recently demonstrated by injecting HDTMA into a confined aquifer; the resultant sorptive barrier effectively immobilized naphthalene in a simulated contaminant plume (Burris and Antworth 1992).

C. SCOPE

We have divided our objective into four main areas of investigation: 1) the binding of QUATs to soils and subsoils, 2) the biocompatibility of QUATs with NOC-degrading microorganisms, 3) the biostability of QUATs once exchanged onto natural soils and clays, and 4) the bioavailability of the immobilized NOC to indigenous microorganisms. The experimental approach, results and discussion for each of these objectives will be outlined in detail in the subsequent sections of this report.

SECTION II

QUAT BINDING

A. SUMMARY

Both static and kinetic studies were conducted to probe the sorption and desorption of HDTMA, a model quaternary ammonium compound, by a subsoil via both cation exchange and hydrophobic bonding. In the kinetic study, we found that adsorption of HDTMA (via cation exchange plus hydrophobic bonding) by a Na-saturated soil is more rapid than Na^+ release, indicating a slower transfer of some HDTMA adsorbed via hydrophobic bonding to cation exchange sites.

The static studies include adsorption and desorption of HDTMA, electrophoretic mobility and the degree of dispersion of soil clay as influenced by HDTMA adsorption. The adsorption study suggested that HDTMA can be strongly held on exchange sites of soil clay at a loading level less than 75% of the cation exchange capacity of the soil (CEC), indicated by the cation selectivity coefficients of Ca-HDTMA exchange in the range between 10^9 to 10^7. The HDTMA loading beyond this level may result in HDTMA adsorbed on low energy sites with a cation exchange coefficient of Ca-HDTMA exchanged smaller than 10^4, and thus a higher residual HDTMA concentration in soil solution. The desorption of HDTMA from the subsoil by 0.5-25 m*M* $CaBr_2$ and $CaCl_2$ solutions reveals that HDTMA adsorption via cation exchange sites

is irreversible. Hydrophobic bonding, which is not significant in low HDTMA loading levels, becomes a competing mechanism for HDTMA adsorption at HDTMA beyond 75% CEC, and can lead to a total HDTMA adsorption up to 340% CEC. Unlike the HDTMA adsorbed on exchange sites, the HDTMA adsorbed via hydrophobic bonding can be easily desorbed, especially at low ionic strengths. The adsorption mechanisms also influence the colloidal stability of the soil clays. HDTMAs adsorbed on exchange sites promote the clay flocculation by tail-tail interaction between adsorbed HDTMA, whereas the HDTMAs adsorbed via hydrophobic bonding develop a positive charge on clay surfaces indicated by reversal of electrophoretic mobility of the soil clays and lead to clay dispersion suggested by the increased turbidity of soil suspension.

The above results suggested that HDTMA-soil clay complexes are very stable as long as we limit the adsorption of HDTMA via hydrophobic bonding. The suggested ways to minimize the HDTMA adsorption via hydrophobic bonding is lowering ionic strength and controlling the companion anions (e.g. using HDTMA Cl instead of HDTMA Br).

Appendix A contains reprints of two published articles describing in detail our studies of QUAT binding to soils. The references are:

1. Xu, S. and S.A. Boyd. 1994. Cation exchange chemistry of hexadecyltrimethylammonium in subsoils containing vermiculite. Soil Sci. Soc. Amer. J. 58:1382-1391.

2. Xu, S. and S.A. Boyd. 1995. Cationic surfactant sorption of a vermiculite subsoil via hydrophobic bonding. Environ. Sci. Technol. 29:312-320.

SECTION III

BIOCOMPATIBILITY

A. ORGANISMS WHICH APPEAR TO BE COMPATIBLE WITH QUATS

1. Introduction

The germicidal properties of quaternary ammonium compounds have been known for more than 60 years and as such these compounds are used widely as antibacterial agents for cleaning surfaces, medical instruments and disinfecting skin. In addition, the surfactant properties of QUATs has led to their inclusion in laundry detergents, as fabric softeners and in products such as shampoos and hair conditioners. Most studies on the antibacterial nature of these compounds have focused on individual microorganisms of medical importance or on aquatic microorganisms, as a result of the use of QUATs in many household products. However, very little information is available on the toxicity of these compounds to soil microorganisms. In addition, complex formation between these QUATs and suspended particles is known to reduce the toxicity of these compounds and in soil this will be a major contributing factor to the toxicity of these compounds.

To investigate the bactericidal nature of HDTMA to soil and aquifer microorganisms, changes in the microbial density and diversity following HDTMA amendment of Eielson aquifer material and Marlette A soil were quantified. In addition, bacteria which

survived the HDTMA amendment of Marlette A soil were characterized with respect to their sensitivity to HDTMA. Furthermore, since a potential application of the soil modification technology is the management of gasoline-contaminated groundwater plumes, we have investigated the susceptibility of toluene, naphthalene and phenanthrene degrading bacteria to DODMA and HDTMA.

2. Methods

Changes in the microbial density and diversity of soils following treatment with HDTMA were quantified as follows. Marlette A soil and Eielson aquifer material slurries (10 g of soil, 50 ml of phosphate-buffered saline, PBS; Smibert and Krieg 1981) were exposed to HDTMA at 50% of the soil CEC for 1 hour with shaking. Subsequently, the slurries were blended briefly and serially diluted in PBS. Soil dilutions (0.1 ml) were spread onto the surfaces of four different agar media. Bacterial colonies growing on the agar plates were counted after 2 to 3 weeks of incubation in the dark at 25 °C. In addition, the number of different colony types appearing on each agar medium was quantified as an indication of the bacterial diversity in each soil. Viable plate counts and the colony diversity on these plates were compared to results from soil samples not amended with HDTMA. Eleven colonies with different colony morphologies from the HDTMA amended samples were isolated and subsequently purified for further analysis with respect to surfactant sensitivity.

The sensitivity of these strains and several well known bacterial species to HDTMA was determined by incubating washed cell suspensions in PBS solutions with a range of HDTMA concentrations. Following incubation for 1 hour, the cells were serially diluted in PBS and dilutions were spread onto the surface of nutrient agar or PTYG agar (Nye et al. 1994) and incubated for 1 week at 25°C. A comparison of the colony counts between untreated samples and HDTMA-treated samples provided an estimate of the percent survival. Linear regression of the percent survival as a function of the log of the HDTMA concentration was used to obtain estimates of the LC_{50} value for each isolate, or the HDTMA concentration at which 50% survival occurred.

Additionally, aromatic degrading bacteria were isolated from untreated Tyndall aquifer material and their sensitivity to HDTMA and DODMA was determined. To isolate toluene, naphthalene and phenanthrene degrading bacteria, Tyndall aquifer material (6 g) was added to 60 ml of a basal salts medium (BSM, Kolenc et al. 1988) and shaken for 30 minutes on a reciprocating shaker to disperse the bacteria. The sample was then serially diluted in the same medium. One dilution series was set up for each carbon source. Each dilution series then received 50 ppm toluene, 10 ppm naphthalene or 2.5 ppm phenanthrene and the vials were immediately sealed. The vials were shaken on a rotary shaker at 150 rpm and incubated at room temperature. The medium also

contained resazurin as an indicator of metabolic activity. Vials were assumed to contain active degrading populations if the indicator changed from blue/purple to pink/red (DiGeronimo et al. 1978). Vials with the pink/red coloration were then opened and the contents streaked onto basal salts agar medium and incubated under toluene or naphthalene vapors to isolate the respective degraders. Phenanthrene degrading bacteria were isolated by first streaking the positive dilution vials onto a soil extract agar and then spraying the developed colonies with a phenanthrene solution to produce a crystalline layer of phenanthrene over the surface of the agar. Colonies capable of degrading phenanthrene were identified by the formation of clearing zones in this layer.

Selected colonies were isolated, purified and their ability to grow on the respective substrate confirmed by repeated transfer and growth in BSM plus toluene, naphthalene or phenanthrene as the only carbon source. The sensitivity of 15 strains (five of each group) to HDTMA and DODMA was assessed as the minimum growth inhibitory concentration (MIC). The previous method for determining the percent survival was not utilized since several of these strains clumped in PBS and dilutions for viable plate counts were either over- or under-estimated so that dilutions yielding statistically countable colony numbers were missed.

Overnight cultures were harvested by centrifugation and resuspended in 1/10 PTYG broth tubes (10 ml final volume) to an

absorbance at 650 nm (A650) of 0.09 to 0.11. HDTMA or DODMA (0.1 to 0.2 ml of isopropanol stocks) was then added to the tubes to yield a range of concentrations and the initial A650 values were measured. All tubes were incubated at 25°C for 5 to 6 days at which time growth was assessed by measuring the A650 of each culture. Because of DODMA precipitation in 1/10 PTYG at concentrations as low as 20 µM and HDTMA precipitation at 50 µM, turbidity could not be used as the sole criterion of growth, and growth was confirmed by transfer to nutrient agar or 1/10 PTYG plates. A wide concentration range was initially used and this was progressively narrowed to more accurately determine the MIC value for each culture. The results are presented as a range from the lowest concentration supporting growth to the next highest concentration not supporting growth. Surfactant concentrations were not adjusted for the loss of surfactant as a result of precipitation or sorption. Therefore, the values provided are conservative estimates of the toxicity of HDTMA and DODMA to these strains.

3. Results

The general toxicity of HDTMA to soil microorganisms was observed by both a reduction in bacterial numbers and diversity (Table 1). On average, a 15 to 9 fold reduction in the number of culturable bacteria in the Eielson and Marlette soils, respectively occurred following an HDTMA treatment for 1 hour. The microbial diversity was reduced by approximately 50% in both

TABLE 1. CHANGES IN MICROBIAL DENSITY AND DIVERSITY IN EIELSON AND MARLETTE A SOILS FOLLOWING HDTMA AMENDMENT.

Soil	Media[a]	Treatment[b]	# Colony Types	# Bacteria[c]
Eielson	1	control	14	7.73×10^5
	1	HDTMA	4	4.00×10^4
	2	control	12	2.86×10^5
	2	HDTMA	5	1.66×10^4
	3	control	17	9.73×10^5
	3	HDTMA	7	6.73×10^4
	4	control	5	5.46×10^5
	4	HDTMA	2	5.21×10^4
Marlette	1	control	18	2.09×10^6
	1	HDTMA	9	5.27×10^5
	2	control	14	9.39×10^5
	2	HDTMA	6	5.91×10^4
	3	control	20	3.14×10^6
	3	HDTMA	9	7.21×10^5
	4	control	5	6.09×10^5
	4	HDTMA	3	5.16×10^4

[a]Media 1 corresponds to Nutrient, media 2 to PTYG, media 3 to 1:20 PTYG and media 4 to Tap Water.
[b]Controls were incubated in PBS, HDTMA treatments at 50% saturation of the CEC, each for one hour prior to plating.
[c]Represents the number of viable bacteria per gram of soil, dry weight.

soils, as estimated by the number of distinct colony morphologies.

Of the eleven bacteria isolated from these plates, seven were spore-formers, and one to two isolates contained a Gram-negative cell wall structure (Table 2). Most of the soil isolates were similarly sensitive to HDTMA with LC_{50} values between 28 and 61 μM (Table 2). The variation in HDTMA sensitivity among the soil bacteria was illustrated by the presence of two extreme LC_{50} values of 7 μM for isolate J and 514 μM for isolate D. The HDTMA sensitivities of the pure cultures fell within the range observed for the soil isolates. Furthermore, it appeared that the Gram-positive bacteria were generally more resistant to HDTMA than the Gram-negative bacteria.

Several interesting trends in the toxicity of HDTMA and DODMA to the aromatic degrading bacteria were apparent (Table 3). HDTMA was clearly more toxic than DODMA to these bacteria; with MIC values for HDTMA usually below 2 μM HDTMA. Naphthalene degrading bacteria were possibly more resistant to the effects of both DODMA and HDTMA than either the toluene or phenanthrene isolates. The toluene and phenanthrene degrading isolates were apparently equally resistant to either surfactant, with one exception. The phenanthrene degrading isolate, TPM 44, was the most sensitive strain to both DODMA and HDTMA. Additional studies with narrower concentration ranges are needed to confirm these observations.

TABLE 2. CHARACTERISTICS OF BACTERIA ISOLATED FROM HDTMA-AMENDED MARLETTE A SOIL AND HDTMA LC_{50} VALUES FOR THESE ISOLATES AND SEVERAL TYPE STRAINS.

Isolate		Gram Reaction	Spore Formation	HDTMA Toxicity LC50 (uM)
Soil isolates				
	A	+	+	44.0
	C	+	+	46.0
	D	+	+	514.0
	F	+	+	61.0
	H	+	+	54.0
	J	-	-	7.2
	O	+	+	46.0
	P	+/-	-	51.0
	W	+	-	51.0
	X	+	-	28.0
	Y	+	+	28.0
Pure Cultures				
	Pseudomonas putida	-	-	4.0
	Micrococcus luteus	+	-	53.0
	Rhodococcus rhodochrous	-	-	37.0
	Arthrobacter globiformis	+	-	7.0
	Alcaligenes sp. NP-Alk	-	-	8.5

TABLE 3. MINIMUM INHIBITORY GROWTH CONCENTRATIONS (MICs) TO HDTMA AND DODMA FOR AROMATIC HYDROCARBON DEGRADING BACTERIA ISOLATED FROM TYNDALL AQUIFER MATERIAL.

Carbon Source	Isolate	Surfactant MIC ranges (uM) DODMA	HDTMA
Toluene			
	TTM 13	10<x<100	0.1<x<1
	TOL 5	20<x<50	0.25<x<0.5
	TOL 8	10<x<100	0.75<x<1
	TOL 12	<10	0.1<x<1
	TTM 16	10<x<100	>0.75
Naphthalene			
	TNM 2	50<x<100	0.75<x<1
	TNM 34	> 100	10<x<25
	TNM 48	25<x<50	<1
	TNM 55	> 100	10<x<25
	TNM 65	> 100	10<x<25
Phenanthrene			
	TPM 3	10<x<100	0.5<x<0.75
	TPM 23	50<x<60	1<x<2
	TPM 32	1<x<10	1<x<2
	TPM 44	<20	<0.1
	TPM 46	<10	0.1<x<1

4. Discussion

Treatment of the Marlette A soil or Eielson aquifer soil with HDTMA at a level equivalent to 50% saturation of the CEC led to substantial reductions in both bacterial numbers and diversity compared to unamended samples (Table 1). These results are evidence of the general toxicity of HDTMA to indigenous soil microorganisms. In addition, this bactericidal effect of HDTMA was exerted quite rapidly as the soils were exposed to HDTMA for 1 hour.

The isolation of bacteria surviving the HDTMA treatment resulted in larger numbers of spore-forming bacterial types, indicating that population shifts after soil modification with HDTMA may be towards spore-formers (Table 2). Bacterial spores represent a dormant resting state in the life-cycle of certain bacteria. These spores are characteristically resistant to various harmful agents, such as heat, drying, radiation, acids and chemical disinfectants (Brock 1979). Examples of both the presence (as reported in Lawrence 1970) and absence (Chaplin 1951) of sporicidal activity by various QUATs have been reported. In this study, the recovery of predominantly spore-forming bacteria from the HDTMA-amended soils suggests any bacterium present as a spore within the soil will probably survive a surfactant treatment. The isolation of non-spore forming bacteria from the HDTMA-treated soils suggests that these bacteria are either able to resist the bactericidal effects of

HDTMA or were protected within the interior of bacterial microcolonies or within micropores of the soil matrix.

Bacterial sensitivities to QUATs vary with the species and type of bacterium. Bacteria with a high degree of resistance to HDTMA, such as isolate D, appear to be a rare occurrence in soils (Table 2). Chaplin (1951) has shown that resistance to QUATs is present to a variable degree among cells of a single culture as indicated by sigmoidal survival curves. He was able to propagate the last survivors and show that these survivors had an increased resistance to the disinfectant than the original population. Isolate D may therefore represent one strain of a soil species with an increased resistance to HDTMA. Similarly, sporadic colony growth of armoatic degrading strains was observed after exposure to QUATs, indicating the presence of a few cells with increased resistance to QUATs.

Gram-positive bacteria were usually more resistant to HDTMA with some exceptions (Table 2). For example, *Arthrobacter globiformis* was the most sensitive of the Gram-positive bacteria studied and the HDTMA sensitivity of *Rhodococcus rhodochrous*, a Gram-negative bacterium, was similar to the HDTMA sensitivities of the remaining Gram-positive bacterial strains. In contrast, Gram-negative bacteria were found to be more resistant to the bactericidal effect of QUATs than Gram-positive bacteria (Davis 1990; Gilbert and Al'Taae 1985; Hueck et al. 1966). The

increased resistance of Gram-negative cells to QUATs is thought to be the result of an extra outer layer of lipoproteins and lipopolysaccharide material (Chaplin 1952; Davis 1990). In other studies, a wide range of germicidal activities was noted against a variety of bacteria, such that conclusions regarding the susceptibility of Gram-positive versus Gram-negative bacteria to QUATs were not possible (as reported in Lawrence 1970). These differences may be the result of widely different test systems, that most of the strains utilized are of medical importance, and that the composition of the surfactant mixtures may not have been consistent.

The susceptibility of representative aromatic degrading bacterial isolates to DODMA and HDTMA also showed that certain bacterial populations may be slightly more susceptible to the bactericidal effects of surfactants. The toluene and phenanthrene degrading bacteria were apparently equally sensitive to HDTMA and DODMA, while naphthalene degraders were slightly more tolerant of these QUATs (Table 3). The reasons for this difference are not presently known, but these results suggest that bacteria utilizing complex substrates may not necessarily have a decreased resistance to QUATs.

Aquifer bacteria were found to be more resistant to the dialkyl C_{18} QUAT, DODMA, than the monoalkyl C_{16} QUAT, HDTMA (Table 3). Similarly, Hueck et al. (1966) observed that mono-quaternaries having two or three alkyl chains have lower

biological activities than QUATs with one alkyl chain, except when the chain length is eight or fewer carbons. In addition, the toxicity of dodecyltrimethylammonium bromide generally decreased as substitution of the hydrogen atoms by ethyl radicals increased until all three hydrogens were replaced (Valko and Dubois 1945). Our results are consistent with these findings and with other studies which indicate that dialkyl QUATs are less toxic than monoalkyl QUATs (as reported in Boethling 1984).

The differential toxicity of DODMA and HDTMA implies that structural differences between QUATs affect the relative toxicity of the QUAT to microorganisms. In general, a balance between the hydrophilic and hydrophobic nature of the QUAT may be important, since these properties affect the surface activity of the QUAT (Attwood and Florence 1983). Valko and Dubois (1945) observed that the germicidal effectiveness of surfactants was affected by the general molecular structure, the type of N-alkyl radicals and the length of the paraffin chain. In addition, with respect to surfactant toxicity, the length of the higher aliphatic radical and the nature of the lower alkyl substituent on the nitrogen were interdependent. Furthermore, as the molecular weight of the QUAT increases, the water solubility and thus availability of the surfactant to cells decreases along with the ability to penetrate membranes (James et al. 1987). DODMA also has a greater tendency than HDTMA to precipitate in protein rich media and to sorb to surfaces, which effectively decreases its aqueous, and thus,

effective concentration. All of these factors have been hypothesized to explain the reduced bactericidal activity of DODMA compared to HDTMA.

We have investigated the susceptibility of soil and aquifer bacterial populations to the surfactants HDTMA and DODMA. In addition, we further investigated the toxicity of these compounds to a small number of bacteria isolated from these sites either prior to or after a surfactant treatment. Our results have shown the general toxicity of HDTMA to indigenous soil and aquifer bacteria, as indicated by reductions in bacterial density and diversity following a 1 hour treatment. In addition, the general toxicity of both HDTMA and DODMA was further indicated by the similar sensitivities of the isolates to these surfactants. Two types of bacteria were apparently more resistant to HDTMA or both HDTMA and DODMA: the Gram-positive and naphthalene degrading bacteria, respectively. Since only a limited number of bacteria were compared (11 Gram-positive, 5 Gram-negative, and 5 naphthalene degrading bacteria) these results are only preliminary and may not reflect the susceptibility of soil and aquifer bacteria in general. In addition, no effort was made to consider the sorption or precipitation of either surfactant in the test systems and thus the actual effective bactericidal concentration. As a result, the LC_{50} values and MIC ranges represent conservative estimates of the toxicity of HDTMA and DODMA to the soil and aquifer bacterial isolates. Furthermore,

the biocompatibility of these surfactants to either anaerobic or chemolithotrophic bacteria within the soils was not investigated. At present very little is known about the toxicity of QUATs to anaerobes (Boethling 1984) or to chemolithotrophs, although there is some evidence that nitrification in wastewater and river water is severely inhibited by QUATs (Boethling 1984; Tubbing and Admiraal 1991).

One prominent result of this study was the reduced toxicity of DODMA compared to HDTMA. This result along with other bactericidal studies on homologous series of different classes of QUATs suggest that alternate QUATs may be identified which are more compatible with indigenous soil and aquifer bacteria.

B. EFFECT OF QUATs ON AROMATIC SUBSTRATE MINERALIZATION

1. Introduction

The biocompatibility of HDTMA and DODMA to indigenous microbial populations in soils and aquifer materials was determined by investigating the ability of these microbial populations to mineralize various substrates.

A part of this material has been published previously and a reprint of that work appears in Appendix B. The reference is:

1. Nye, J.V., W.F. Guerin, and S.A. Boyd. 1994. Heterotrophic activity of microorganisms in soils treated with quaternary ammonium cations. Environ. Sci. Technol. 28:944-951.

2. Methods

The impact of HDTMA and DODMA on the heterotrophic activities of indigenous soil microorganisms was assessed by measuring the mineralization of ^{14}C-labelled substrates in soil slurries. Soil slurries consisted of 10 g of soil plus 50 ml of sterile water, PBS or BSM (Tyndall aquifer material only). Slurries were amended with HDTMA or DODMA at concentrations equivalent to exchange with 0, 30, 50 or 70% of the soil's CEC. The surfactants were added to the soils as a percentage of the soil CEC to permit exchange with the soil and to reduce the possibility of aqueous surfactant remaining in solution. The actual amounts of surfactant added on a per gram of soil basis are shown in Table 4. HDTMA and DODMA stock solutions were prepared in the respective diluent for each soil type and all DODMA stock solutions were sonicated to disperse the DODMA. Solutions of ^{14}C-labelled substrates plus sufficient unlabelled substrate were prepared in the respective diluent to achieve the final desired concentration and activity (5×10^3 dpm/ml) in the slurries. Substrates utilized in this study were glucose, salicylate, 2,4-dichlorophenoxy acetic acid (2,4-D), toluene, naphthalene and phenanthrene. Table 4 summarizes the experimental conditions used in this study by substrate, soil and surfactant, since not all combinations of these three variables were analyzed.

TABLE 4. SUMMARY OF SUBSTRATES, SOILS AND SURFACTANTS USED IN THE MINERALIZATION ASSAYS TO INVESTIGATE THE EFFECTS OF THE SURFACTANTS ON SOIL MICROBIAL POPULATIONS.

Substrate	[Substrate] (ug/ml)	Soil	Surfactant	[Surfactant] (% soil's CEC)	[Surfactant] (mg/g soil)
Glucose					
	200	Marlette A	HDTMA	0; 30; 70	0; 12.57; 29.33
	50	Marlette A	DODMA	0; 30; 50	0; 21.77; 36.28
	50	Marlette B	DODMA	0; 30; 50	0; 24.23; 40.38
	200	Eielson	HDTMA	0; 10; 30; 70	0; 2.92; 8.75; 20.41
	50	Oshtemo B	DODMA	0; 30; 50	0; 7.19; 11.99
Salicylate					
	5	Marlette A	HDTMA	0; 30; 70	0; 12.57; 29.33
	5	Eielson	DODMA	0; 50	0; 25.24
2,4-D					
	10	Marlette A	HDTMA	0; 30; 70	0; 12.57; 29.33
Toluene					
	5	Eielson	HDTMA	0; 10; 30; 70	0; 2.92; 8.75; 20.41
	15	Marlette B	HDTMA	0; 30 50 70	0; 14.0; 23.33; 32.6
	15	Marlette B	DODMA	0; 30 50 70	0; 24.23; 40.38
	15	Oshtemo B	HDTMA	0; 30; 50	0; 4.15; 6.92
	15	Oshtemo B	DODMA	0; 30; 50	0; 7.19; 11.99
	15	Tyndall	HDTMA	0; 30 50 70	0; 0.44; 0.73; 1.02
	15	Tyndall	DODMA	0; 30 50 70	0; 0.76; 1.26; 1.77
Naphthalene					
	1	Eielson	HDTMA	0; 10; 30; 70	0; 2.92; 8.75; 20.41
	1	Eielson	DODMA	0; 50	0; 25.24
	2	Tyndall	DODMA	0; 30; 50	0; 0.76; 1.26
Phenanthrene					
	0.5	Eielson	HDTMA	0; 10; 30; 70	0; 2.92; 8.75; 20.41
	1	Tyndall	HDTMA	0; 30 50 70	0; 0.44; 0.73; 1.02
	1	Tyndall	DODMA	0; 30; 50	0; .076; 1.26

3. Results

The soils utilized in this study were selected to give a range of textural classes, CECs, and clay contents (Table 5). These soils differed primarily in their CECs, which were relatively high for the Marlette and Eielson soils and low for the Oshtemo and Tyndall soils. "The Marlette A and B horizon soils had similar CECs with the clay mineral fraction contributing relatively more of this capacity in the B horizon and the organic fraction contributing more in the A horizon. The Eielson aquifer material was a fairly fine-grained soil due to its high silt content. It also contained appreciable organic carbon and a relatively high CEC considering its subsurface origin." (Nye et al. 1994). The Oshtemo B and Tyndall aquifer materials were fairly coarse due to their high sand contents, however the Tyndall aquifer material contained a more uniform size distribution of sand particles than the Oshtemo soil and lacked larger pebbles and small stones found in the Oshtemo soil (the latter items were removed before use). Additionally, both aquifer materials had been previously exposed to jet fuel contamination, while the other soils are mainly of agricultural origin.

A summary of the biocompatibility of HDTMA toward the indigenous heterotrophic microbial populations in the various soils is shown in Table 6. Representative mineralization curves for some of the substrates presented in this table are provided

TABLE 5. CHARACTERISTICS OF SOILS USED IN THE MINERALIZATION ASSAYS.

Soil	% Sand	% Silt	% Clay	Field Capacity (mL/100 g)	CEC[a] (meq/100 g)	% Organic Carbon
Marlette A	54.9	35.7	9.4	29.2	11.5	2.2
Marlette B	51.5	31.3	17.2	33.9	12.8	0.6
Eielson	32.9	62.8	4.3	N.D.[b]	8.0	0.85
Oshtemo B	89.0	5.0	6.0	N.D.	3.8	0.3
Tyndall	N.D.	N.D.	N.D.	N.D.	0.4	N.D.
Spinks A	75.6	19.6	4.8	N.D.	5.9	1.5
Spinks B	60.2	27.2	12.6	28.6	8.9	0.3
Petroleum-Contaminated	60.0	11.0	29.0	25.1	N.D.	1.9

[a]: CEC = Cation exhange capacity in milliequivalents per 100 grams.
[b]: N.D. = Not determined.

TABLE 6. OVERVIEW OF THE BIOCOMPATIBILITY OF HDTMA TO INDIGENOUS MICROBIAL POPULATIONS IN VARIOUS SOILS.

SUBSTRATE	SOILS	HDTMA EFFECTS
GLUCOSE	Marlette A	Minor lag prior to mineralization
	Eielson	Minor lag prior to mineralization; Extents and rates of mineralization reduced
SALICYLATE	Marlette A	Minor lag prior to mineralization at 70 % level; Extents of mineralization reduced
2,4-D	Marlette A	Extended lag periods; Significant inhibition at 70 % level - less than 2 % mineralized
TOLUENE	Eielson	Reduction in extents and rates of mineralization
	Marlette B	Minor lag prior to mineralization; Reduction in rates and extents of mineralization
	Oshtemo B	Minor lag period at 70 % level; variable inhibition of rates and extents of mineralization
	Tyndall	Reduction in rates and extents of mineralization
NAPHTHALENE	Eielson	Lag prior to mineralization; Reduction in rates and extents of mineralization
PHENANTHRENE	Eielson	Complete inhibition of mineralization
	Tyndall	Minor lag prior to mineralization and reduction of extent of mineralization at 70 % level Inhibition of mineralization rates

in the attached paper by Nye et al. (1994). In most cases, a reduced capability to mineralize the substrate was observed in each soil type for HDTMA additions at 10, 30, 50 or 70% of the soil's CEC. Overall, the response of the microbial community to HDTMA was dependent on the HDTMA treatment level and the structural complexity of the substrate. Generally, the rates and extents of mineralization decreased as the concentration of HDTMA added to a soil increased (see Figures 1 and 2 of Nye et al., 1994). A comparison across the different soils also indicates that the biocompatibility of HDTMA toward the microbial communities was lowest in soils with high exchange capacities. Greater reductions in the rate and extent of toluene mineralization were observed in the HDTMA-amended Marlette B and Eielson soils as compared to HDTMA-amended Oshtemo B and Tyndall soils (Figure 1). Similarly, the toxicity of HDTMA to the phenanthrene degrading microbial population in Tyndall aquifer material (CEC = 0.4 meq/100 g) was significantly lower than the toxicity of HDTMA to this population in the Eielson aquifer material (CEC = 8 meg/100 g soil) (Figure 2 in Nye et al. 1994).

With simple substrates, such as glucose, the inhibitory effects of HDTMA were absent to minor, as evidenced by the absence of or small reductions in the extents of mineralization in Marlette A soil and Eielson aquifer material, respectively (Table 6). With intermediate substrates, such as toluene, salicylate and naphthalene, the degradative capacity was

maintained, despite some minor increases in the lag period prior to mineralization and reductions in the rates and extents of mineralization (Table 6). For the more complex substrates, such as 2,4-D and phenanthrene, significant inhibition of the degradation process occurred as a result of the initial exposure of the soils to HDTMA. The addition of HDTMA at the 30% treatment level significantly increased the length of the lag period prior to 2,4-D mineralization, while less than 2% of the 2,4-D was mineralized at the 70% treatment level (Figure 1 of Nye et al., 1994). Phenanthrene mineralization in Eielson aquifer material was completely inhibited at all HDTMA treatment levels (Table 6). In addition, the treatment of Tyndall aquifer material with HDTMA was inhibitory to phenanthrene degraders, but only slightly decreased the extent of toluene mineralization (Figure 2). The response of indigenous populations in soils to DODMA was relatively minor in comparison to the effects observed with HDTMA (Table 7). Mineralization of glucose, toluene, salicylate and naphthalene was generally unaffected by the addition of DODMA to the various soils. In some cases, such as with toluene in Marlette B or Oshtemo B soils and naphthalene in the Eielson aquifer material, minor reductions in the rate and extent of mineralization were observed following treatment with DODMA (Table 7). As expected, the inhibition of phenanthrene degradation in Tyndall aquifer material by DODMA was significant,

TABLE 7. OVERVIEW OF THE BIOCOMPATIBILITY OF DODMA TO INDIGENOUS MICROBIAL POPULATIONS IN VARIOUS SOILS.

SUBSTRATE	SOILS	DODMA EFFECTS
GLUCOSE	Marlette A	Stimulation of rates and extents of mineralization
	Oshtemo B	No inhibition or minor lag prior to mineralization
SALICYLATE	Eielson	No inhibition
TOLUENE	Marlette B	Extents of mineralization reduced
	Oshtemo B	Effects on extents and rates of mineralization non-reproducible
	Tyndall	No inhibition
NAPHTHALENE	Eielson	Minor reductions in rate and extent of mineralization
	Tyndall	No inhibition
PHENANTHRENE	Tyndall	Minor lag prior to mineralization; inhibition in rates of mineralization Reduction in extents of mineralization at some levels

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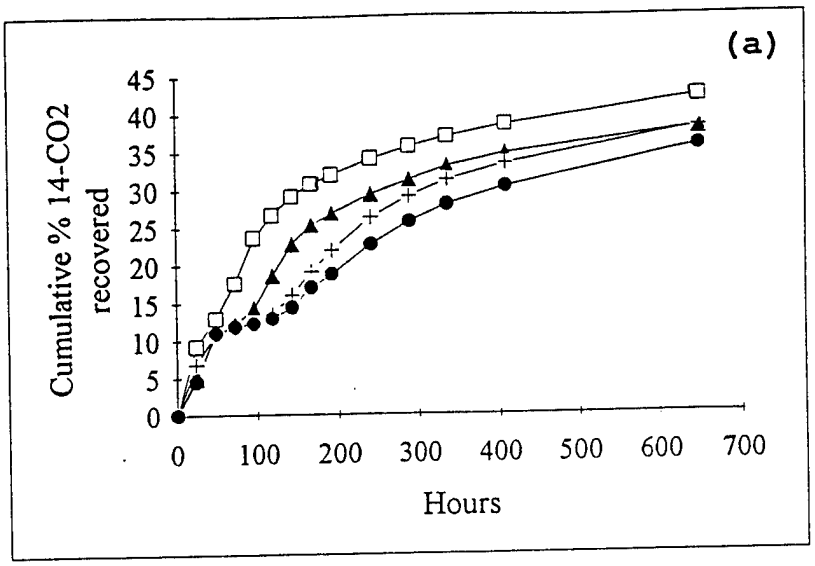

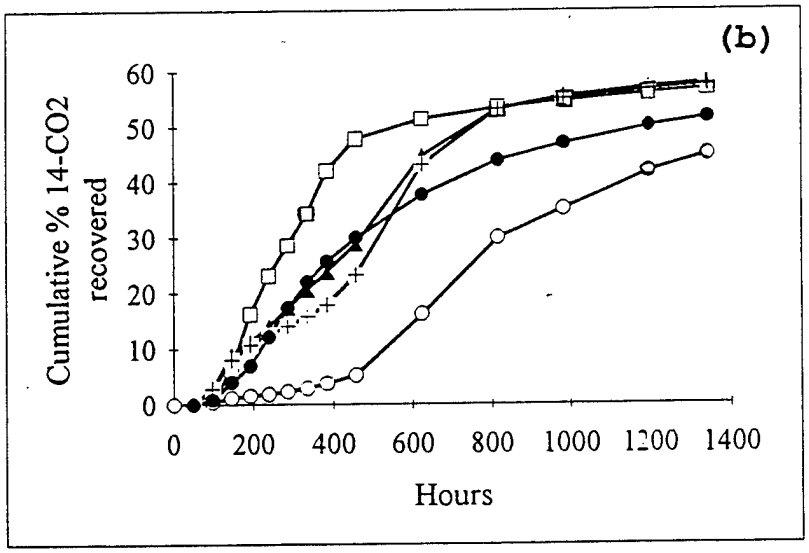

Figure 2. Mineralization of toluene (a) and phenanthrene (b) in Tyndall aquifer material amended with HDTMA at 0%(□), 30%(▲), 50%(+) or 70%(●) of the CEC. Open and closed symbols represent individual slurries that were not averaged at a particular treatment level.

but DODMA did not inhibit the mineralization of toluene or naphthalene (Figure 3).

4. Discussion

The treatment of soils and aquifer matierals with the cationic surfactant HDTMA inhibited the activities of the aerobic, heterotrophic microbial populations. Only with the structurally complex substrates, 2,4-D and phenanthrene, was this inhibition nearly complete. In the latter case, the inhibition of phenanthrene mineralization was primarily a result of the bactericidal effect of HDTMA on a small population of phenanthrene degrading bacteria in the Eielson aquifer (Nye et al. 1994). HDTMA was also presumed to be bactericidal to other microbial populations in the various soils, but with the distinction that a greater number of bacteria survived the HDTMA treatment due to larger population sizes. One result of smaller population sizes following a surfactant treatment was an increase in the length of the adaptation period prior to mineralization. The extended lag periods observed in phenanthrene slurries after surfactant treatment indicated that very few phenanthrene degraders survived the HDTMA treatment and that reestablishment of this population and phenanthrene mineralization were prevented. Since larger populations of glucose, toluene, naphthalene and salicylate degraders survived the surfactant treatment, the length of the lag periods prior to mineralization were slightly increased above that observed in the surfactant-

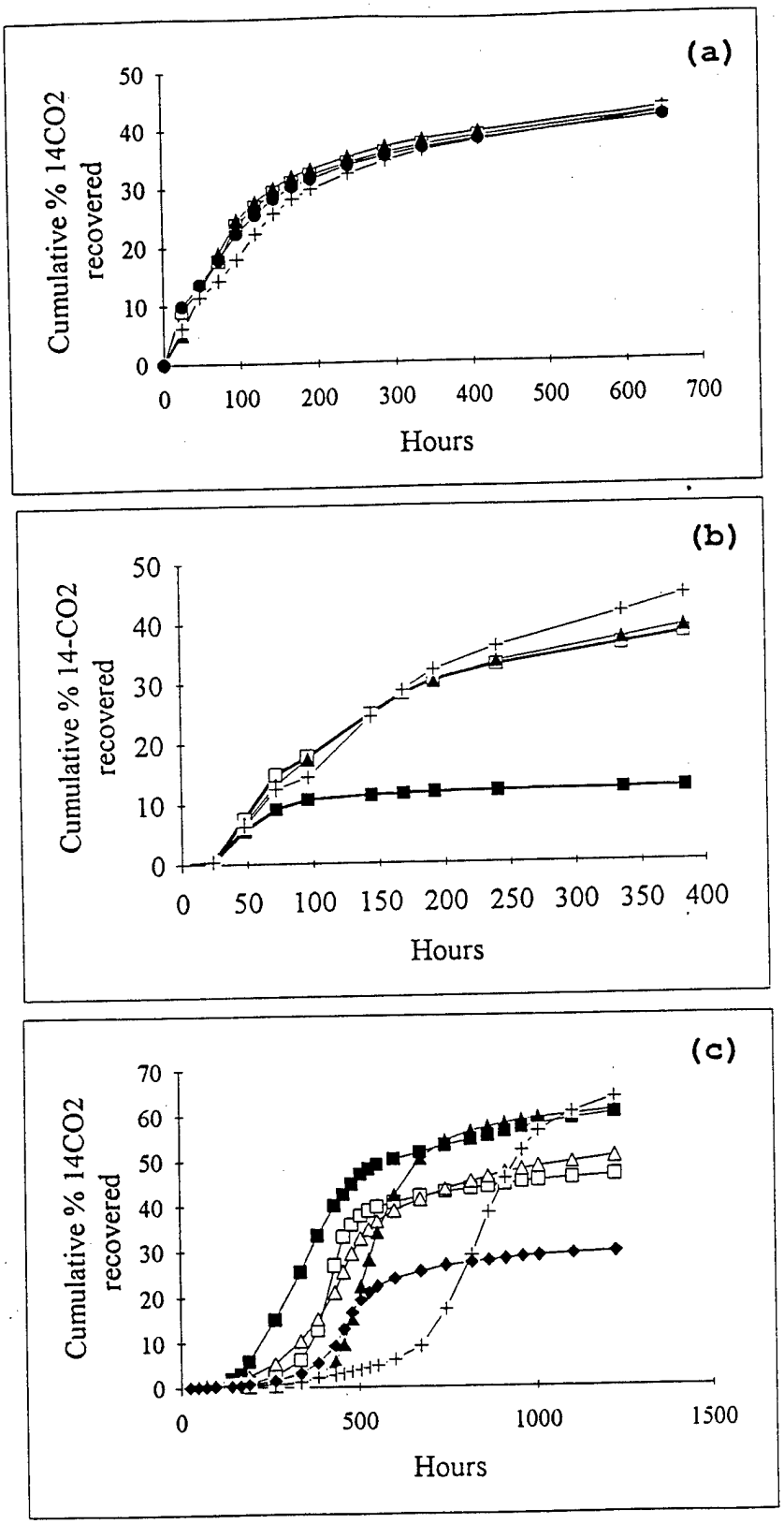

Figure 3. Mineralization of toluene (a), naphthalene (b) and phenanthrene (c) in Tyndall aquifer material amended with DODMA at 0%(□), 30%(▲), or 50%(+) CEC. Open and closed symbols represent individual slurries at a particular treatment level. In panel c, the individual slurries at the 50 % level are represented by(+) and (◆).

free controls. In some cases, in the rate and extent of mineralization are possibly due to changes in the bacterial diversity of degrading populations in these soils. Furthermore, the HDTMA effects on the mineralization patterns may also be caused by an inhibition of microbial metabolism in general.

The compatibility of HDTMA with indigenous microbial populations in soils and aquifer materials was correlated with the CEC of the material. The inhibitory effects of HDTMA were reduced in materials with low CECs in comparison to the inhibitory effects in high CEC materials. The capacity to mineralize phenanthrene was maintained, albeit at a reduced level compared to an untreated sample, in Tyndall aquifer material (CEC = 0.4 meq/100g). In contrast, phenanthrene mineralization was completely inhibited in the Eielson aquifer material with a CEC of 8 meq/100g. These differences can be attributed to a significantly higher initial concentration of free HDTMA added to the Eielson aquifer slurries. Since the free cationic form of QUATs is more toxic than that bound to particles, these results suggest that HDTMA may be more compatible with microorganisms in low CEC soils and aquifer materials. In these materials, lower concentrations of HDTMA can be employed to create the *in situ* sorptive zone, thus reducing the toxic effects of HDTMA to the indigenous microbial populations.

The enhanced biocompatibility of DODMA to soil microbial populations is consistent with our earlier pure culture studies and with the available literature. In fact, the trend observed above for HDTMA was not evident with DODMA, since the inhibitory effects of DODMA on microbial activities were relatively minor compared to HDTMA. It is possible that the availability of DODMA to microbial cells was very limited in the slurries due to its very low water solubility. The addition of DODMA as a colloidal suspension to the slurries probably results in the slow dissolution of DODMA as solution phase DODMA becomes exchanged at sites in the soil matrix. As a result, the concentration of solution phase DODMA never approaches a bactericidal level similar to that observed with HDTMA.

The addition of HDTMA or DODMA to various soils has indicated that microbial activities in these soils will be negatively affected by these surfactants. In most cases, the microbial populations in these soils are able to recover as a result of growth of bacteria which survived the surfactant addition. In a few cases, the numbers of surviving microbial degraders were too small to permit recovery, and thus mineralization potential for phenanthrene and 2,4-D was lost. The HDTMA toxicity was highly correlated with the CEC of the soil or aquifer material, with larger inhibitory effects on microbial mineralization potential observed in higher CEC materials. For both HDTMA and DODMA, the inhibition of mineralization potential

was greater as the structural complexity of the substrate increased. This phenomenon is probably due to a general bactericidal effect on a small population of highly specific degraders. In addition, this population could possess a decreased resistance to the surfactant, although this did not appear to be the case with phenanthrene degrading bacteria isolated from Tyndall aquifer sand (see above). HDTMA was significantly more toxic than DODMA to the indigenous microbial populations responsible for the mineralization of several aromatic and a chlorinated hydrocarbon.

C. SOLUTION VS. SORBED PHASE QUAT TOXICITY

1. Introduction

Cationic surfactants form complexes with dissolved organics, particularly with anionic compounds (Kupfer and Waters 1976) and are strongly sorbed onto solids (Boyd et al. 1988; Larson and Vashon 1983; Games et al. 1982). These properties are thought to be primarily responsible for reducing the toxicity of the cationic surfactants to phytoplankton and heterotrophic bacteria (Lewis and Wee 1983; Tubbing and Admiraal 1991). Furthermore, anionic surfactants in wastewaters play an important role in neutralizing the inhibitory activity of the cationic surfactants, thereby permitting microbial degradation of the cationics at toxic concentrations (Boethling 1984; Games et al. 1982; Sullivan 1983). Studies on the toxicity of cationic surfactants have focused on the use of the surfactants as

disinfectants toward pure cultures of microorganisms and on the exposure of natural aquatic populations in wastewater and aquatic systems. Investigations on the toxicity of QUATs to indigenous microbial soil or groundwater populations are rare. Additionally, measurements of the toxicity of QUATs sorbed to soils or clay materials are important when considering the technology proposed in this report. Since the modified soil zone is to be maintained for long periods for effective groundwater management, it is important to determine the impacts of initial QUAT addition and continued maintenance. In this section, the toxicity of HDTMA as the aqueous cation and HDTMA sorbed to clay or soil was compared using *Pseudomonas putida* ATCC 17484 and indigenous soil microorganisms, respectively.

2. Methods

The toxicity of aqueous HDTMA to *P. putida* was determined by incubating washed cell suspensions in PBS plus 100 µM HDTMA for 1 hour. To determine the toxicity of HDTMA sorbed to clay, smectite clay (CEC = 90 meq/100 g) was added to the HDTMA solutions prior to addition of the *P. putida* cells, to permit sorption of the HDTMA onto the clay. Following treatment, the cultures were serially diluted in PBS and viable plate counts on PTYG or nutrient agar were determined. Colony counts of treated samples were compared to untreated controls to obtain estimates of the percent survival.

The influence of soil-sorbed HDTMA on the mineralization of 2,4-D and naphthalene by indigenous microbial populations was also measured. The mineralization of 2,4-D was followed in Marlette A soil slurries as described earlier. The slurries contained either no HDTMA, HDTMA added as the free cation to achieve 70% saturation of the soil's CEC or an equivalent amount of HDTMA-modified (70% CEC saturation) Marlette B soil. This method was revised by removal of unbound HDTMA in Eielson aquifer material prior to naphthalene mineralization. In this case, sterile aquifer slurries (10 g per 50 ml) were equilibrated (shaken) with HDTMA (50% of CEC) and naphthalene for 24 hours. Following equilibration, unbound HDTMA was scavenged from the flasks by the addition of sterile smectite clay (89 mg) or by the addition of an HDTMA-degrading bacterium. A fourth sample received naphthalene but no HDTMA. After 3 days, the flasks were inoculated with 1 g of non-sterile aquifer material and the mineralization of naphthalene was followed.

3. Results

In pure culture studies with *P. putida* 17484, the toxicity of HDTMA was significantly reduced when the HDTMA was bound to smectite clay prior to the addition of the bacterium (Figure 3 in Nye et al. 1994). Survival of *P. putida* cells was negligible at a concentration of 100 µM HDTMA. On the other hand, the addition of as little as 1.2 mg of smectite clay

completely alleviated the toxicity of the 100 μM HDTMA solution. Even with the addition of 1 mg of clay, bacterial survival was substantially improved compared to the expected viability for the calculated aqueous concentration (data not shown).

Similar reductions in toxicity were observed when HDTMA was introduced prebound to soil or when soils were treated with HDTMA prior to inoculation (Figures 4 and 5 of Nye et al., 1994). HDTMA added as the free cation to Marlette A soil slurries severely inhibited the mineralization of 2,4-D, with less than 2% of the 2,4-D mineralized after 1800 hours (Figure 4 of Nye et al., 1994). In contrast, a significant amount (approximately 50%) of 2,4-D was mineralized in slurries containing HDTMA-modified soil after a shortened lag period (250 hours). By allowing HDTMA to exchange with slurried aquifer material before inoculation, naphthalene mineralization occurred after a shorter lag period and at a faster rate (Figure 5 of Nye et al. 1994) compared to naphthalene mineralization in slurries where HDTMA had been added as the free cation (see Figure 2 in Nye et al. 1994). In the time required to completely mineralize the naphthalene in the HDTMA pretreated slurries (Figure 5 of Nye et al., 1994) less than 10% of the naphthalene had been mineralized in slurries amended with the free cation (see Figure 2 in Nye et al. 1994). HDTMA toxicity was completely eliminated when any remaining unbound HDTMA in the slurries was chemically or biologically scavenged. Naphthalene mineralization curves for

the clay- and bacteria-treated samples were nearly identical to the unamended control (Figure 5 of Nye et al., 1994).

4. Discussion

The comparison between the biocidal activity of clay-sorbed HDTMA and aqueous HDTMA towards strain 17484 indicated that sorption of HDTMA to solids greatly reduces the biocidal activity of HDTMA. Since the initial reaction between the QUAT and the cell involves cell wall/membrane binding, prevention of this step should correspondingly lower the toxicity of the QUAT. Quaternary ammonium cations interact strongly with anions, such as anionic surfactants (Boethling 1984; Sullivan 1983; Games et al. 1982) and phosphates (Chaplin 1951) to form complexes which effectively reduces the toxicity of the QUAT (Chaplin 1951, Sullivan 1983; Games et al. 1982). Removal of the dissolved form of the cation via sorption to solids is also known to reduce or eliminate cation binding to bacterial cells and thus stop the germicidal action of the QUAT. The sorption of ditallow-dimethylammonium chloride (DTDMAC) to suspended solids in river water was shown to reduce the toxicity of DTDMAC to heterotrophic bacteria (Tubbing and Admiraal 1991). Similarly, a reduced toxicity of soil-sorbed HDTMA produced shorter acclimation periods and increased the rates and extents of mineralization for 2,4-D and naphthalene. The reduced toxicity of soil-sorbed HDTMA implies a potential ability to recolonize HDTMA-modified areas in aquifers if the initial HDTMA treatment proves to be detrimental.

The preequilibration of the Eielson aquifer material with HDTMA alone did not completely alleviate HDTMA toxicity (Figure 5 of Nye et al. 1994). This suggests that either dissolved HDTMA was still available to inhibit bacterial cells or that some fraction of the sorbed HDTMA was an effective bactericide. An availability of HDTMA in these slurries was inferred by additional sorption to clay or biodegradation with a concomitant reduction in toxicity (Figure 5 of Nye et al. 1994). Soil sorbed HDTMA may remain accessible if it is sorbed to exterior regions of particles or if several molecules are associated via weak tail-tail interactions. Thus, surfactant toxicity may persist until the cations become immobilized at exchange sites (Nye et al. 1994).

Based on the results from these experiments and the earlier mineralization studies, a chain of events with respect to bacterial toxicity may be inferred. The initial addition of a QUAT to the subsurface will represent the period of greatest toxicity, since the QUAT solution is at a high aqueous concentration and sorption to aquifer material and accessible bacterial cells will occur rapidly. This will be followed by a period in which the effective bactericidal activity of the QUAT is greatly reduced due to the initial sorption and lowered aqueous concentrations. Microbes migrating into the modified-soil zone at this time will have an increased chance of survival. Finally, the continued sorption of unbound surfactant to

additional exchange sites and the movement of exchanged cations from accessible to inaccessible sites on particles will further reduce the effective toxicity of the surfactant to bacteria. At this stage, the QUAT should be at its lowest toxicity and conditions should be highly favorable for recolonization by bacteria in the groundwater.

D. PHYSIOLOGICAL STATE OF SOIL MICROBIAL POPULATIONS

1. Introduction

A variety of microscopic and spectrophotometric methods are available for assessing the metabolic activity of indigenous microbial populations (Atlas and Bartha 1987; Trevors 1984; Bitton and Koopman 1982; McFeters et al. 1995, Zimmerman et al. 1978, Song and Bartha 1990). The metabolic activity of natural soil microbial populations exposed to the cationic surfactants was assessed using the metabolic indicators, fluorescein diacetate and 2 (p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT).

Fluorescein diacetate (FDA) is considered a vital stain because its accumulation and hydrolysis depends upon an intact membrane and active metabolism in bacteria and fungi (McFeters et al. 1995). Within a cell, the FDA is deacetylated by nonspecific esterases resulting in the accumulation of fluorescein within the cell. Using epifluorescence microscopy, cells which fluoresce green are assumed to be metabolically active; or the fluorescein can be extracted with a suitable solvent and the absorbance at 490 nm measured (Song and Bartha 1990).

INT acts as an artificial electron acceptor and is reduced by microbial electron transport systems (ETS) to an insoluble red (non-fluorescent) INT-formazan. The INT-formazan can be detected microscopically as insoluble crystals within actively respiring bacterial or algal cells (Bitton and Koopman 1982; Pearl and

Bland 1982; Trevors et al. 1983). This method is usually paired with a fluorescent counterstain, such as DAPI, fluorescein isothiocyanate or acridine orange, in order to discriminate active bacterial cells from the total bacterial population (Herson et al. 1986; Tabor and Niehof 1982; Zimmerman et al. 1978). Alternatively, bulk soil samples are incubated with INT, the insoluble formazan is extracted with a suitable solvent and the absorbance at 480 nm is measured. The effects of various pesticides, heavy metals and methylene chloride on ETS activity in soils, aquatic samples, sediments and pure cultures has been investigated (Bitton et al. 1984; Chan and Leung 1986; Chendrayan and Sethunathan 1980; Trevors 1982; Trevors 1985; Tu 1981).

2. Methods

In the FDA assay, soil slurries (5 g of Marlette A soil plus 25 ml of phosphate buffer) were incubated in the dark with 20 µg/ml of FDA with or without the addition of HDTMA. The HDTMA was added in an amount equivalent to saturate 30% of the cation exchange sites (12.6 mg of HDTMA/g of soil). The reaction was stopped by the addition of 25 ml of acetone, and the slurries were shaken to extract the fluorescein. The absorbance of the filtrate was then measured at 490 nm. Sterile soil controls consisted of soil which had been sterilized by autoclaving.

To measure the ETS activity of the Marlette A soil, 5 g of soil was amended with either 0.5 ml of distilled water or an aqueous solution of HDTMA. HDTMA amendments of 5, 15 or 23% of

the soil's CEC were used (2.1, 6.29 and 10 mg/g soil, respectively). Next, 0.5 ml of an aqueous INT solution (0.4% w/v) was added and the soil was incubated in the dark at 23 °C. At various times the soil samples were extracted with 20 ml of dimethyl formamide. The absorbance of the filtrate was measured at 480 nm and compared to a standard curve of INT-formazan to quantify the amount of INT-formazan produced.

Alternatively, the bacteria were detached from the soil by shaking 5 g of soil with 20 ml of a Tris buffer for 30 minutes. The soil was allowed to settle and 2 ml of the supernatant containing the bacteria was incubated with the INT (0.5 ml of 0.4% solution) for 2 to 4 hours. Next, 10 µl of the cell suspension was spread over a 1 cm area on a glass slide, air-dried, heat-fixed and then stained with fluorescein isothiocyanate. The bacterial cells were examined with simultaneous epifluorescence and transmitted light to obtain a count of the total microbial cells and the number of cells that were metabolically active, respectively. Control samples did not receive INT.

3. Results

Fluorescein-diacetate could not be used as an indicator of microbial activity in the presence of HDTMA, since color development occurred in sterile soil controls and in soil-free controls containing HDTMA. Color development may have occurred

via a reaction between the positively charged HDTMA and the negatively charged FDA.

Next, the use of INT as an indicator of the microbial activity in Marlette A soil exposed to HDTMA was assessed. The ETS activity in unamended Marlette A soil slowly increased over the 24 hour incubation period (Figure 4). A transient drop in activity at the 10 hour time point occurred in this sample and also in two soil samples amended with HDTMA. The nonlinear nature of the curves meant that rates of INT reduction for each soil sample could not be calculated. In soil treated with HDTMA at 5, 15 or 23% of the soil's CEC, the ETS activity of the soil was generally scattered around the value measured for the unamended soil treatment. These results suggested that HDTMA did not inhibit the metabolic activity of the soil microorganisms. An exception to this trend occurred with samples incubated for 24 hours. In this case, the ETS activity in the presence of HDTMA at any concentration was inhibited. The fluctuations in the ETS measurements in the soil samples may have been caused by poor recovery of the INT-formazan from the soil as suggested by low absorbance values (maximum around 0.06 to 0.12). Difficulties in recovering the formazan were confirmed by adding a known concentration of INT-formazan to soil and measuring the amount recovered. Recoveries were between 50 and 85% efficient.

In order to circumvent the recovery problem, soil bacteria were detached from the soil, incubated with INT and then the number of metabolically active cells determined

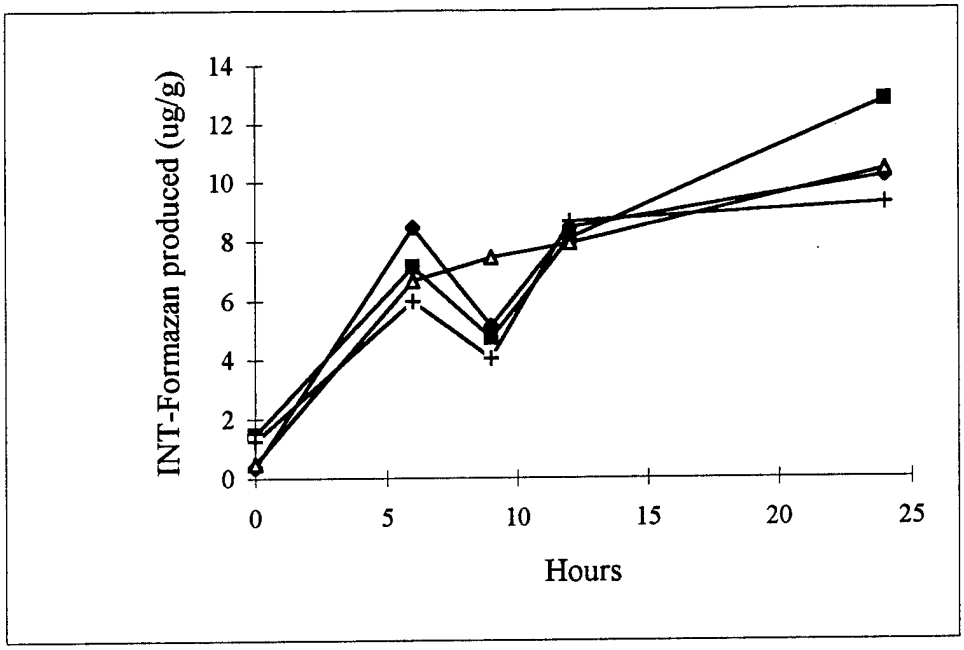

Figure 4. ETS activity in Marlette A soil (■) or in Marlette A soil amended with HDTMA at 5%(+), 15%(◆) or 23%(△) of the soil's CEC.

microscopically. Provided that the bacteria within the soil were metabolically active, these active cells should contain red formazan granules when visualized with transmitted light. The results from this technique were also unreliable. In Marlette A soil incubated with INT, the proportion of bacteria containing INT-formazan granules and thus metabolically active was about 12%. However, in control samples about 5% of the population was counted as containing formazan granules, even with the absence of INT, suggesting that artifacts or soil debris were being misinterpreted as formazan granules.

4. Discussion

Of the three methods we used to assess microbial activity changes in response to surfactant treatment, only the INT assay appeared to be suitable. Data could not be obtained with the FDA assay or the INT-FITC direct count technique due to interference from HDTMA or the presence of false positives, respectively. Metabolic activity measurements were possible with the INT assay in the absence or presence of HDTMA, but the results were highly variable due to poor recovery of the INT-formazan. The poor recovery was the result of the irreproducible extraction of the formazan itself from soil. Although, low microbial activities in the soil or a low availability of INT to microorganisms as a result of sorption of the cation to soil would also produce low formazan levels. As a result of the lack of sensitivity and reproducibility with the INT assay, the effect of HDTMA on the

metabolic activity of soil microorganisms could not be predicted with certainty. Thus, we decided to continue using more reliable measurements of bacterial activity or viability in the presence of the surfactants, such as the mineralization assays and pure culture toxicity assays.

SECTION IV

BIOSTABILITY

A. INTRODUCTION

An important consideration in the development of a coupled immobilization-biodegradation technology is the stability of the soil- or clay-bound QUAT against microbial attack. Although QUATs are used as anti-microbial agents (Gilbert and Al-Taae 1985; Lawrence 1950), microbial adaptation to and mineralization of QUATs have been observed in environments with a history of exposure to these compounds (Federle and Ventullo 1990; Shimp et al. 1989; Sullivan 1983; Ventullo and Larson 1986). Furthermore, organisms with the ability to utilize HDTMA as a sole carbon and energy source have recently been described (van Ginkel et al. 1992; Mueller et al., unpublished data).

In this study, exchange complexes were prepared using ^{14}C-labelled HDTMA and several clay minerals (illite, smectite and vermiculite) and soils. The biostability of these complexes was evaluated under unsaturated conditions in several soils and in saturated soil slurries.

The biostability of DODMA added as an aqueous solution of the free cation or exchanged onto whole soils was evaluated in soils under unsaturated conditions.

B. MATERIALS AND METHODS

1. Chemicals

Hexadecyltrimethyammonium bromide (HDTMA), 99% pure, was

obtained from Sigma Chemical Co., St. Louis, MO. ^{14}C-HDTMA-Br, labelled on the terminal (C-16) carbon of the hexadecyl chain, was obtained from Moravek Biochemicals, Inc. (Brea, CA) and has a radiochemical purity of 97%. DODMA was obtained from Eastman Kodak Company, Rochester, NY and ^{14}C-DODMA, labelled on the terminal carbon of one of the C-18 chains, was purchased from California Bionuclear.

2. Soils

Marlette A horizon (sandy loam) and B horizon (loam) and Spinks A horizon (loamy sand) and B horizon (sandy loam) soils were collected from agricultural fields near East Lansing, Michigan. An additional soil was obtained from northern California which contained approximately 500 *u*g/kg (ppb) total petroleum hydrocarbons following a bioremediation project utilizing anionic surfactants. After collection, the soils were air dried for one hour, screened to pass a 2 mm sieve, and stored at 4 °C . Soils were characterized for CEC (Rhoades 1982), organic carbon content, particle size distribution (Grigal 1973), and field capacity (Ritchie and Crum 1989). Soil properties are summarized in Table 5.

3. Clays

The following reference clays were used in this study: Montana illite, South Carolina vermiculite, and Wyoming smectite. Organo-clay complexes were prepared by reacting each clay with

HDTMA-Br. A mixture of ^{14}C-labelled and unlabelled HDTMA was dissolved in deionized water and added in an amount equal to 30, 70, or 100% of the CEC of the clay. The clay solutions were thoroughly mixed and equilibrated to allow complete exchange. The clays were collected by centrifugation, rinsed with deionized water and freeze-dried.

Additionally, HDTMA or DODMA-modified Marlette A and B horizon soils were prepared at 70% of the CECs. Aqueous QUAT solutions spiked with ^{14}C-QUAT were added to soil slurries. Exchange was considered complete after mixing for four hours. Soils were collected by centrifugation, washed once with deionized water and allowed to air dry.

4. Biostability of HDTMA-clays in Unsaturated Soils

The biostability of HDTMA-illite, smectite, and vermiculite in the Marlette and Spinks soils was assessed in four experiments monitored for 2.5 and 4 months (short-term) and 12 and 18 months (long-term). In these experiments, 25 g (oven-dry basis) of one unmodified soils were placed in duplicate flasks along with 100 mg of HDTMA-illite, smectite, or vermiculite prepared at 30, 70, or 100% of its CEC. The soil and HDTMA-clay were then thoroughly mixed and brought to 70% field capacity with deionized water. Additionally, the unmodified soils (no HDTMA-clay added) were amended with a ^{14}C-labelled HDTMA solution containing a mass of HDTMA equal to that added to the HDTMA-smectite amended soils ("control" treatment). Flasks were capped

evolution was monitored for 7.5 months. The biostability of the ^{14}C-DODMA soils was assessed under unsaturated conditions for 8 months. 100 mg of either ^{14}C-DODMA-Marlette A or B soil was added to 25 g of unmodified soil from the same horizon and incubated at 70% field capacity.

6. Biostability of HDTMA-Clays in Saturated Soil Systems

The stability of the ^{14}C-HDTMA-clays under saturated soil conditions was assessed in experiments in which 50 mL of deionized water were added to 25 g of Marlette A horizon or PHC soil. 100 mg of either HDTMA-illite, smectite, or vermiculite (100% CEC) were added to duplicate biometer flasks. Two additional "control" flasks received a ^{14}C-labelled HDTMA solution (no HDTMA-clay) added after measuring its initial radioactivity. Biometers were placed on a rotary shaker to maintain an aerobic environment and were periodically opened for sampling.

7. Biostability of DODMA Added as the Free Cation

In this study, aqueous ^{14}C-labelled DODMA solutions were prepared which contained DODMA in an amount equivalent to that exchanged onto 100 mg of smectite clay at 30, 70, or 100% clay CEC saturation. These solutions were then thoroughly mixed with 25 g of unmodified soil (Spinks or Marlette A and B horizons) and incubated at 70% field capacity. Sampling and analysis procedures are the same as previously mentioned for biometer studies.

8. Mass Balance Determination

At the termination of the ^{14}C-HDTMA-clay incubations in the unsaturated Marlette and Spinks A and B horizon soils, soil subsamples from representative flasks were analyzed to attempt a material mass balance. Soils were air dried and homogenized before combustion of three 0.5-gram portions in a Harvey Biological Material Oxidizer model OX-300 (R.J. Harvey Instrument Corporation). $^{14}CO_2$ was collected and analyzed by LSC to determine the total ^{14}C-HDTMA remaining in the soil. Knowing the initial ^{14}C-HDTMA added and the total $^{14}CO_2$ collected during the 12 month incubation, the results of the oxidation analysis were used to attempt a mass balance by summing the ^{14}C-HDTMA remaining in the soil and that evolved as $^{14}CO_2$.

C. RESULTS

The results from the long-term incubations in Marlette and Spinks A and B horizon soils, designed to test the biostability of HDTMA-exchanged clays, are summarized in Table 8. Occasionally, one of the duplicate treatments mineralized significantly greater amounts of HDTMA than the corresponding replicate, and these values are presented individually when the variation was greater than 25%. In the 12 and 18 month experiments, HDTMA degradation began immediately in some flasks, while others exhibited a lag of up to 9 months before significant mineralization commenced. Subsequent experiments, with 2.5 and 4

TABLE 8. CUMULATIVE $\%^{14}CO_2$ RECOVERIES FROM THE MINERALIZATION OF HDTMA ADDED DIRECTLY TO SOIL AS THE FREE CATION OR AS A HDTMA-CLAY.

Soil Type	HDTMA Loading (%CEC of Clay)	Incubation Length (months)	Free-HDTMA‡	HDTMA-Illite	HDTMA-Smectite	HDTMA-Vermiculite	Sterile Controls
Marlette A (Sandy Loam)	30	12	2.0, 33.9	3.3	<1.0, 2.1	2.7	
	70	12	1.7	3.4	<1.0	<1.0, 30.0	
	100	12	1.3	2.2	>1.0, 11.9	<1.0	
	100	18	1.0, 7.1	3.5, 29.4	2.1	1.1, 2.9	
	70	2.5	53.0	54.3	38.8		
Marlette B (Loam)	30	12	<1.0	<1.0, 2.3	<1.0	<1.0	
	70	12	<1.0	1.0	<1.0	<1.0	
	100	12	<1.0	<1.0, 8.3	<1.0	<1.0	
	100	18	<1.0	1.0, 4.5	<1.0	1.0, 30.2	
	70	2.5	56.1	50.0	35.9		
Spinks A (Loamy Sand)	30	12	4.9, 31.3	6.3	1.1	3.7	
	70	12	3.6, 36.1	5.4	2.1,4.6	2.3, 24.1	
	100	12	2.0, 43.6	3.9	2.0	1.5	
	100	18	1.1	2.7	1.9, 20.4	1.8, 2.5	
	70	4	26.0	40.9	26.2		1.1
Spinks B (Sandy Loam)	30	12	<1.0	<1.0, 37.6	<1.0, 12.6	1.1	
	70	12	<1.0	1.4	<1.0	<1.0	
	100	12	<1.0	<1.0	<1.0	<1.0, 36.1	
	100	18	1.0	1.3	1.6	1.0	
	70	4	48.0	44.0	35.8		<1.0
Petroleum-Contaminated	70	6.2	6.2	14.9, 25.0	6.4	7.0	

† Single values reported are the averages of duplicate flasks. When the duplicates varied by >25%, the individual values are presented in the table.

‡ HDTMA was added directly to the soils as an aqueous solution in an amount equivalent to that added to the HDTMA-smectite treated soils.

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months incubation, exhibited at most, a 3 week lag prior to HDTMA mineralization to CO_2.

In general, free or clay-bound HDTMA was resistant to biodegradation in both the A and B horizons of two representative agricultural soils (Marlette and Spinks) in the long-term experiments. Less than 5% of the added ^{14}C-HDTMA was recovered as $^{14}CO_2$ in 85% of the 128 biometer flasks used in the Marlette and Spinks soil incubations. Mass balance calculations indicated that 61% to 68% of the added label could be accounted for in flasks with > 20% conversion to $^{14}CO_2$ and > 99% was recovered in flasks with < 1% mineralization of the added ^{14}C-HDTMA. Incubations in the petroleum-contaminated soil under unsaturated conditions showed that less than 7% of the HDTMA was mineralized when added as the free cation or when bound to smectite or vermiculite. HDTMA-illite, however, was less stable and replicate flasks evolved 14.9 and 25% of the ^{14}C-HDTMA as $^{14}CO_2$ after a five-month incubation (Table 9).

Additional biostability experiments with the HDTMA-clays incubated in the Marlette and Spinks soils yielded substantially different results. In two experiments with incubations of 2.5 and 4 months, greater than 25% of the ^{14}C-HDTMA exchanged on the clays tested was recovered as $^{14}CO_2$ (Table 8). Approximately 1% of the ^{14}C-HDTMA was evolved as $^{14}CO_2$ in sterile (autoclaved) controls, indicating that the losses observed in the treatment flasks were due to biotic rather than abiotic processes.

TABLE 9. CUMULATIVE $\%^{14}CO_2$ RECOVERIES FROM THE MINERALIZATION OF HDTMA ADDED TO SOIL DIRECTLY AS THE FREE CATION OR AS A HDTMA-CLAY IN SOIL SLURRIES.

Soil Type	HDTMA Loading (%CEC of Clay)	Incubation Length (months)	Free-HDTMA‡	HDTMA Illite	HDTMA-Smectite	HDTMA-Vermiculite
Marlette A	100	4.8	<1.0	1.9	<1.0	<1.0
Petroleum-Contaminated	100	5.3	1.1, 2.0	<1.0, 1.5	<1.0, 2.8	4.9, 19.5

† Single values reported are averages of duplicate flasks. When the duplicates varied by >25%, the individual values are presented in the table.

‡ HDTMA was added directly to soils as an aqueous solution in an amount equivalent to that added to the HDTMA-smectite treated soil.

Greater HDTMA stability was observed in aerobic saturated soils (Table 9) compared to incubations at levels below saturations (e.g., 70% of field capacity, Table 8). In the PHC-soil slurry, 7 of 8 biometers showed less than 5% HDTMA mineralization during a five-month period. In the Marlette A horizon soil slurries, very little mineralization (< 2%) occurred during a five-month incubation (Table 9).

The percentage of HDTMA mineralized when added in an unbound form in both the Marlette and Spinks A horizon soils was generally greater than for any of the HDTMA-clay complexes tested. In B horizon soils, however, both clay-bound and free HDTMA was extremely stable in the long-term experiments, with less than a few percent recovered as CO_2 in most cases. In the short-term studies, we observed similar biostabilities in both A and B horizon soils.

The stability of HDTMA complexes with illite, smectite, and vermiculite were compared. HDTMA-smectite complexes were the most stable with less than 1% of the HDTMA mineralized in more than half of the biometers containing the organo-clay and an agricultural soil in long-term incubations. In the PHC-soil only 6.4% of the added HDTMA bound to smectite was recovered as CO_2 despite prior surfactant exposure and the high density of indigenous petroleum-degrading microbes (10^5 to 10^6 degraders/g soil). The HDTMA-vermiculite complexes were less stable, and HDTMA-illite was the most susceptible to degradation. In the 2.5

and 4 month experiments, this same general trend was evident, although the biostabilities of the free HDTMA and the HDTMA exchanged onto illite and smectite were greatly reduced.

HDTMA-soils were prepared to estimate the relative stability of HDTMA bound to cation exchange sites of soil organic matter predominant in A horizon soils as compared to that bound to the cation exchange sites of clays predominant in B horizon soils. Approximately 75% of the CEC in the Marlette A soil (11.5 meq/100 g) is due to exchange sites on organic matter. In the Marlette B horizon, 75% of the CEC (12.8 meq/100 g) is due primarily to illite and vermiculite clay minerals. Two experiments were monitored for 7.5 and 9 months to assess the biostability of ^{14}C-HDTMA-Marlette A and B horizon soils in unmodified Marlette soils. Generally HDTMA bound to the A horizon soils was more stable than HDTMA either bound to the B horizon soil or added directly as the free cation. There was less microbial activity (greater biostability) toward either HDTMA-Marlette A or B in the Marlette B horizon soil as compared to the A horizon soil.

The stability of HDTMA-Marlette A or B soil was increased in biometers which contained the larger ratio of modified to unmodified soil (4 g HDTMA-modified to 10 g unmodified soil, Table 10). In this experiment, HDTMA-Marlette B soil was the most stable with 13.4% of the label recovered as $^{14}CO_2$. HDTMA-Marlette soil and HDTMA added as the free cation were less stable, but their stability was greater than observed when added

TABLE 10. CUMULATIVE $\%^{14}CO_2$ RECOVERIES FROM THE MINERALIZATION OF HDTMA ADDED TO SOIL DIRECTLY AS THE FREE CATION OR AS A HDTMA-SOIL.

Soil Type	HDTMA Loading (% CEC of Soil)	Incubation Length (months)	Free-HDTMA‡	HDTMA-Marlette A	HDTMA-Marlette B	HR§ Free-HDTMA	HR HDTMA-Marlette A	HR HDTMA-Marlette B
Marlette A	70	7.5	65.3	58.8	95.7	26.1	37.6	13.4
	70	9	68.8	59.7	66.5			
Marlette B	70	7.5	70.0	53.2	72.9			
	70	9	57.2	51.8	57.1			

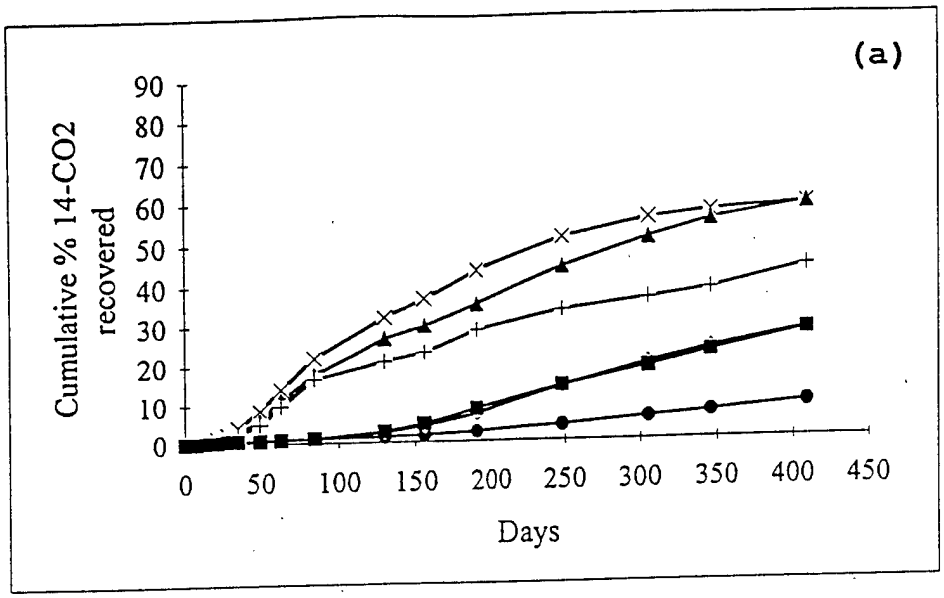
† Values in the table represent the average of duplicate flasks.
‡ HDTMA was added directly to the soil as the free cation in an amount equal to the average of that exchanged onto the Marlette soils.
§ HR refers to high ratio between the added HDTMA-modified soils and the unmodified soils in which they were incubated.

at a lower ratio (100 mg HDTMA-modified to 25 g unmodified soil). Plate counts of bacteria culturable on nutrient agar were an order of magnitude less for biometers containing the HDTMA-Marlette soil (3.5×10^6 cells/g soil) as compared to unmodified Marlette A soil with no exposure to HDTMA (3.5×10^7) cells/g soil). However, Marlette A soil with HDTMA in an amount equivalent to that exchanged onto the 4 grams of modified soil added directly to the soil as the free cation, showed an additional 35% decrease in the culturable population (2.25×10^6 cells/g soil).

The addition of free DODMA to agricultural soils in an amount equivalent to that exchanged onto 100 mg of smectite clay at 30, 70 or 100% saturation of the clay's cation exchange capacity will be referred to by the actual concentration of DODMA added as 0.68, 1.59, and 2.27 mg DODMA/g soil, respectively. Table 11 relates these concentrations to the percentage of the cation exchange capacity for each soil, along with some properties of the soils used in this study. In terms of the CEC of each soil, the amount of DODMA added is very low ranging from 0.84% of CEC saturation in Marlette B soil amended with 0.68 mg DODMA/g soil to 6.1% of CEC saturation in Spinks A soil treated with 2.27 mg DODMA/g soil. Degradation of DODMA occurred at each test concentration after a moderate (20 to 50 days) to extended (100 to 200 d) lag period in Marlette A and Spinks A soils and Marlette B and Spinks B soils, respectively (Figure 5). In the

TABLE 11. CHARACTERISTICS OF SOILS USED IN THIS STUDY AND THE AMOUNT OF DODMA ADDED AS A PERCENTAGE OF A SOIL'S CEC.

Soil	CEC (meq/100g)	Organic Carbon (%)	Percentage of cation exchange capacity for DODMA additions at 0.68 mg/g	1.59 mg/g	2.27 mg/g
Marlette A	11.5	2.2	0.94	2.19	3.13
Marlette B	12.4	0.6	0.84	1.97	2.81
Spinks A	5.9	1.5	1.83	4.28	6.1
Spinks B	8.9	0.3	1.21	2.83	4.05



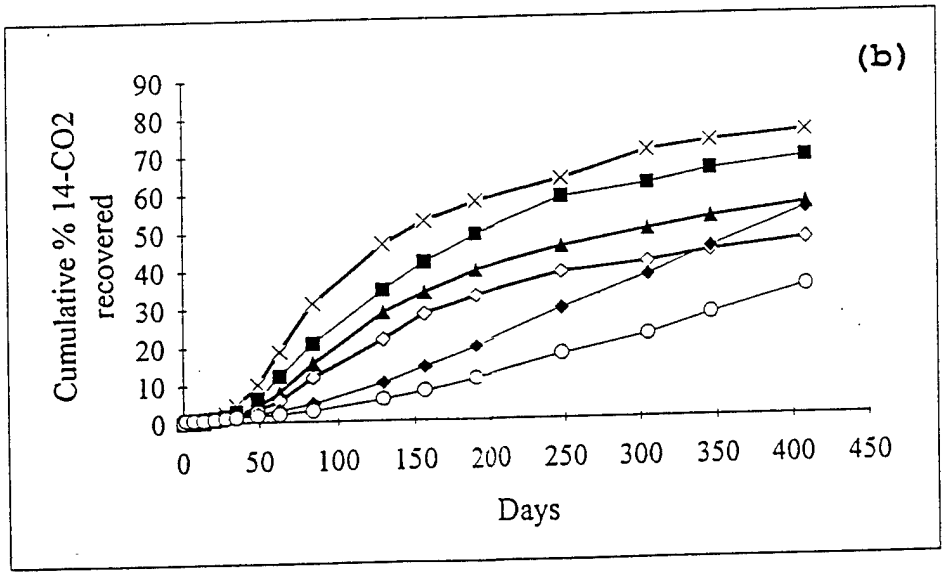


Figure 5. a. Biostability of DODMA added as a free cation in Marlette A (x,▲,+) and B (●,◊,■) soils. DODMA added as the equivalent amount bound to 100 mg of smectite clay and exchanged at 30 % (x;●), 70 % (▲;◊) or 100 % (+;■) of the CEC of the clay. b. Biostability of DODMA added as a free cation in Spinks A (x,▲,◊) and Spinks B (◆,■,o) soils. DODMA was added as an equivalent amount bound to 100 mg of smectite clay and exchanged at 30 % (x;■), 70 % (▲;◆) or 100 % (◊;o) of the CEC of the clay.

Marlette A soil an initial rapid phase of DODMA degradation was followed by a slower phase of degradation. Degradation of DODMA in Spinks A soil followed an exponential pattern, as did the degradation of DODMA in Spinks B soil amended with 0.68 mg DODMA/g soil. In Marlette B and Spinks B soils the degradation of DODMA was linear once DODMA utilization commenced and the rate was apparently slower than in the A horizon soils. As the treatment level of DODMA increased the extent of DODMA utilized generally decreased in Marlette A and both Spinks soils. In the Marlette B soil, the degradation of DODMA was similar and at a faster rate at the two highest treatment levels compared to the lowest treatment level. Furthermore, the rate and extent of DODMA degradation was usually much lower in the B horizon soils than in the A horizon soils.

The degradation of DODMA exchanged onto Marlette A or Marlette B soil was determined in the respective unmodified soil under simulated field conditions. In this case, sterile DODMA-modified soil was mixed with non-sterile soil. The various additions of DODMA-modified soil or free DODMA to each soil are explained in Table 12. The data for the amount of DODMA degraded is an approximation based on the theoretical amount of the ^{14}C-labelled DODMA added that would have exchanged with the soil.

DODMA-modified Marlette A soil was rapidly degraded following a lag of approximately 20 days (Figure 6). After 150 days of incubation, approximately 50% of the label had been

TABLE 12. SUMMARY OF CONDITIONS USED TO INVESTIGATE THE BIOSTABILITY OF DODMA-MODIFIED MARLETTE A OR MARLETTE B SOIL.

Treatment	Modified Soil	Additions of Aqueous DODMA	Unmodified soil
A	Marlette A: 100 mg	N.A.*	Marlette A: 25 g
B	N.A.	5.7 mg	Marlette A: 25 g
C	Marlette B: 100 mg	N.A.	Marlette B: 25 g
D	N.A.	5.7 mg	Marlette B: 25 g
E	Marlette A: 4 g	N.A.	Marlette A: 10 g
F	Marlette B: 4 g	N.A.	Marlette B: 10 g

* N.A.: not applicable

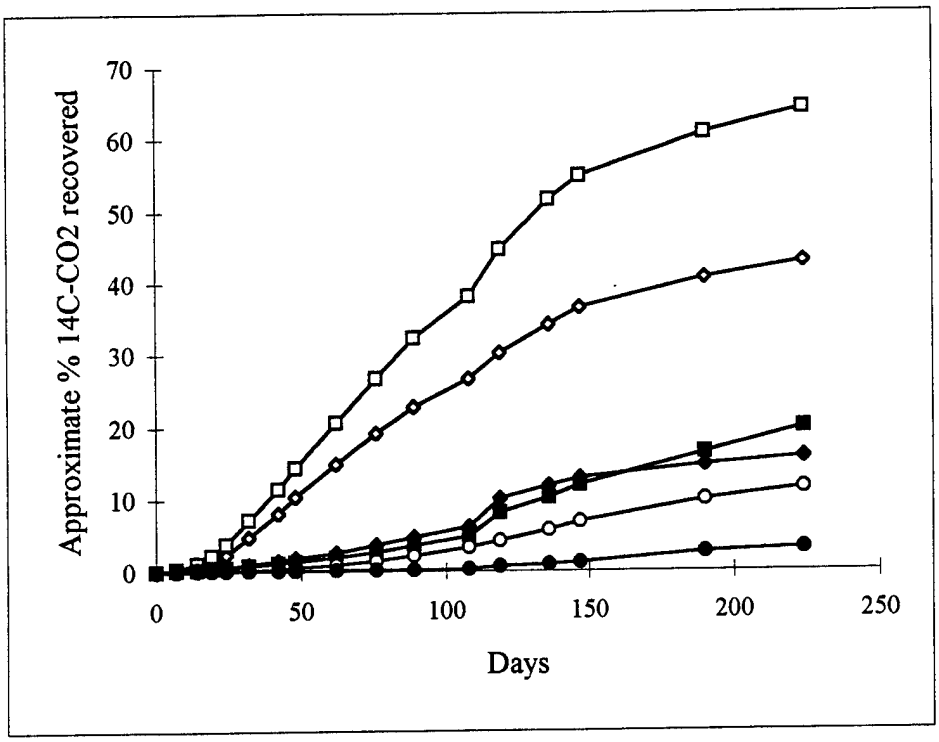


Figure 6. a. Biostability of DODMA-modified Marlette A soil (100 mg) in Marlette A soil (□) or DODMA-modified Marlette B soil (100 mg) in Marlette B soil (■). The biostability of a free cationic addition of DODMA was also followed in Marlette A (◊) and Marlette B (♦) soils. b. Biostability of DODMA-modified Marlette A soil (4 g) in Marlette A soil (○) or DODMA-modified Marlette B soil (4 g) in Marlette B soil (●).

converted to CO_2 and there was no indication that the rate of degradation was slowing. A significantly slower rate of degradation of Marlette B exchanged DODMA in Marlette B soil was observed and about 10% of the DODMA was converted to CO_2. The mineralization rates of soil-exchanged DODMA were slightly faster than those of DODMA initially added as a free cation. With the addition of 4 g of DODMA-modified soil to 10 g of unmodified soil, the length of the lag period approximately doubled and the rate of degradation decreased significantly. The amount of DODMA degraded after approximately 150 days of incubation was less than 10% in Marlette A and 2% in Marlette B (Figure 6).

D. DISCUSSION

The labelled carbon in the ^{14}C-HDTMA used in this study was located on the terminal carbon of the hexadecyl group (away from the ammonium headgroup) to provide a conservative estimate of the biostability of HDTMA-clays in incubations with soils. Several soil types, specifically a sandy loam from the A horizon (Marlette), a loam soil from the B horizon (Marlette), a loamy sand A horizon soil (Spinks), and a sandy loam B horizon soil (Spinks), as well as a petroleum-contaminated soil, were chosen to represent a range of field conditions based on different CECs and organic carbon and clay contents. These factors are known to influence the diversity, density and metabolic activity of the indigenous microbial population (Atlas and Bartha 1987) and possibly the availability of the QUAT.

HDTMA-clay biostability experiments at 70% field capacity in Marlette and Spinks soils gave differing results when comparing $^{14}CO_2$ evolution data from short-term and long-term experiments. Overall, the mineralization of HDTMA in soils and subsoils was low in the long-term studies. Typically, less than 5% of the HDTMA was evolved as $^{14}CO_2$. However, in the short-term experiments, HDTMA bound to clay minerals exhibited less stability as $^{14}CO_2$ recoveries were 40.9% to 54.3% from ^{14}C-HDTMA-illite and 26.2% to 38.8% from ^{14}C-HDTMA-smectite. $^{14}CO_2$ recovered from ^{14}C-HDTMA added directly as the free cation ranged from 26.0% to 56.1%.

There are several possible explanations for these differing results. HDTMA-modified clays used in all experiments were from the same preparation. Potentially, the terminal carbon of the C-16 chain may have undergone a chemical transformation in storage, rendering the terminal carbon less resistant to microbial attack, although this is unlikely. The unmodified soil used in the short-term experiments was collected at a later date than that used in the long-term experiments. Perhaps the microbial diversity had changed and contained microorganisms with the ability to more easily utilize the terminal carbon of the hexadecyl chain of HDTMA. However, attempts to isolate cultures of HDTMA-degrading microorganisms from the biometers were unsuccessful.

Results of this study indicate that HDTMA is generally biologically stable and the extent of mineralization is dependent on the nature of exchange sites where HDTMA is bound and on environmental conditions. The observed differences in the biostability of HDTMA comprising HDTMA-clays are related to the location of the exchange sites on the clay minerals. Biostability experiments have shown that HDTMA bound internally (e.g., smectite) is more stable against microbial attack than that which is externally bound (e.g., illite). Of the three HDTMA-clays tested, HDTMA-smectite is by far the most stable, both chemically and biologically due to the intercalation of HDTMA. A smectitic clay, fully exchanged with HDTMA, has an interlayer d_{001} spacing of approximately 1.8 nm (18 A). An average bacterial cell with a diameter of 1.5 *um* is approximately 750 times larger than this and cannot move into the inner layers of the clay and gain direct access to the exchanged HDTMA. HDTMA-illite, on the other hand, consists primarily of mineral sheets which have no internally exchanged HDTMA due to the complete collapse of the clay interlayers when occupied by K^+ ions. Hence, in illite, all HDTMA is externally bound, apparently giving microbes direct access to the QUAT, resulting in decreased biostability. The results with HDTMA-vermiculite are more difficult to interpret. Despite having HDTMA intercalated, it was mineralized to a much greater extent than

observed for HDTMA-smectite. A recent study of HDTMA exchange onto vermiculitic subsoils indicated that HDTMA occupies predominantly the outermost exchange sites in vermiculite, i.e., near the edges of the clay particles (Xu and Boyd 1994). Because vermiculite is a limited expanding clay, the most accessible sites for HDTMA exchange are likely those near-edge sites; once HDTMA occupies these sites, it may block further access to additional exchange sites in the interior of the clay particle. As a result, only a small portion of the HDTMA may be intercalated. This may explain why the biostability of vermiculite-bound HDTMA is somewhat more than illite-bound, yet less than that exchanged onto smectite, which is a fully expanding clay.

HDTMA, free or pre-bound to clays and free DODMA, was more resistant to microbial degradation when introduced into subsurface (B horizon) soils than in surface (A horizon) soils. Generally, the B horizon soils contain less microbial diversity and a smaller population of microbes. Also, lower concentrations of organic and inorganic growth substrates in the subsurface soils result in reduced microbial activity, and therefore, greater stability of the QUATs.

Long term biostability studies at 70% field capacity indicated that HDTMA added directly to surface soils was less resistant to degradation than that added in a clay-bound form. When added as the free cation to surface soils, HDTMA binds to

the exchange sites of soil clays and soil organic matter, the latter being more abundant in the surface soils we studied. The avidity of binding of HDTMA to SOM is likely less than to clay sites, therefore HDTMA added in the free form to surface soils was more readily mineralized than that added in a pre-bound state to clays. Our conclusion was supported by the similar biostabilities of HDTMA added either as the free cation or clay-bound to B horizon soils where the SOM levels are low and the cation exchange sites are derived primarily from clays.

The relative stability of HDTMA bound to soil organic matter versus the mineral matter fraction of soils was assessed by monitoring the mineralization of HDTMA bound to Marlette A and B horizons in the same unmodified soils. Despite the differences in the source of the CEC (approximately 75% of the CEC is derived from SOM in the Marlette A horizon soil as compared to 25% in the B horizon), equal rates and extents of mineralization were observed. Differences in the clay mineralogy and experimental conditions employed may provide an explanation for this seeming paradox. Smectite-bound HDTMA showed the greatest biostability in our studies and Marlette soil does not contain smectite clays. Also, the unmodified soil used in these biometers was collected at the same time as the soil used in the short-term HDTMA-clay biostability experiments which also showed an increase in mineralization of the terminal HDTMA carbon. The stability of the HDTMA-modified Marlette A and B horizon soils was increased

by increasing the ratio of HDTMA-modified to unmodified soil. These results are encouraging because they more closely represent conditions in a field application. Although plate counts showed that the bacterial population decreased initially in these treatments we expect growth of the resistant strains and repopulation of the site by additional bacteria carried by groundwater flow.

We demonstrated that HDTMA is generally biologically stable in surface and subsurface soils; typically less than 5% of the HDTMA was mineralized in 12 and 18 month incubations. The extent of mineralization depends on the nature of the clay cation exchange sites and on the environmental conditions. Clay-bound HDTMA appeared to be more biologically stable than HDTMA bound predominantly to SOM. HDTMA-smectite complexes offer the greatest biostability, followed by HDTMA-vermiculite and -illite complexes. Internally-exchanged HDTMA (e.g., smectite) is the most stable because microbes are unable to gain direct access to the HDTMA. HDTMA was more susceptible to microbial attack in A horizon soils than in B horizon soils, presumably due to the higher numbers and diversity of microorganisms in the surface horizon. The observed long-term stability of HDTMA in subsoils is important because one of the potential applications of this soil modification technology is the underground injection of HDTMA to create a subsurface sorptive zone that may be useful in

reducing the migration of contaminants (Burris and Antworth 1992).

Compared to HDTMA, DODMA was significantly less recalcitrant to microbial degradation in these soils. In contrast, dialkyl dimethyl QUATs were generally found to be more resistant to microbial degradation than alkyl trimethyl QUATs in aquatic systems (Boethling 1984, Swisher 1987; Federle and Ventullo 1990; van Ginkel and Kolvenbach 1991; Dean-Raymond and Alexander 1977; Larson and Vashon 1983). Using a homologous series of alkyltrimethyl and dialkyldimethyl QUATs and a HDTMA degrading bacterium, van Ginkel and Kolvenbach (1991) observed that for both classes as the alkyl chain length increased, the resistance of the QUAT to biodegradation increased. Furthermore, the resistance to degradation increased significantly with the increase in the number of alkyl chains linked to the nitrogen atom (van Ginkel and Kolvenbach 1991). More specifically, rate constants of the order of 0.1 to 0.2 day^{-1} were determined for the mineralization of HDTMA and stearyltrimethylammonium chloride (STAC; $R=C_{18}$) in river water compared to rate constants of 1 $week^{-}$ for distearyldimethyl-ammonium chloride (DSDMAC; $R_1= R_2=C_{18}$) (Larson and Vashon 1983). Similarly, DSDMAC was degraded at a significantly slower rate than STAC in wastewater ponds, with 13-16% and 32-50% of the ^{14}C-label recovered as CO_2, respectively, over a period of 80 days (Federle and Ventullo 1990). If we assume that the greater toxicity of HDTMA compared to DODMA is

responsible for the recalcitrance of HDTMA, then the soil degradation studies shown here confirm this assumption. However, while the initial dose of free HDTMA may have been toxic and prevented degradation, biodegradation of the pre-bound and reduced toxic form of HDTMA did not occur. Together with the reports discussed above, these results suggest that toxicity differences alone cannot explain these differences in the biostability of HDTMA and DODMA in soil or in aquatic samples.

Our initial study of the addition of DODMA as the free cation to four soils determined that indigenous soil microbial populations could adapt and degrade DODMA. This adaptation occurred at a moderate (20 to 50 days) to slow (50 to 100 days) rate and was dependent on the soil type. In addition, the rate and extent of DODMA degradation varied with the soil type; greater biostability was observed in B horizon soils.

Similar trends in the pattern of DODMA degradation were observed when soil-exchanged DODMA was incubated in the Marlette soils. DODMA exchanged onto the Marlette B soil was significantly more stable than DODMA pre-bound to Marlette A soil. Furthermore, in both the A and B horizon soils a significantly lower rate and extent of exchanged DODMA was degraded when a larger proportion of the soil mixture consisted of modified-soil. These results suggest that once DODMA becomes bound to soil its bioavailability is significantly reduced. It was not possible to determine half-lives of DODMA degradation in

either of these two studies, since the degradation of DODMA in some treatments did not reach a plateau (maximum amount degraded) within the allowed incubation periods. This information would be helpful in predicting the stability of the modified soil zone and the necessity for multiple treatments to maintain the modified zone.

A key issue with respect to the biostability of the modified soil zone, is to what extent bacterial metabolism of the QUAT reduces NOC sorption. By radiolabeling the terminal carbon atom on one of the C_{18} chains we have only investigated the conversion of that carbon atom to CO_2, providing a conservative estimate of the biostability of DODMA in soils. The fate of both C_{18} chains, the methyl groups or the formation of metabolic intermediates was not investigated. Radiolabeling of the terminal (C_{18}), the proximal (C_1) and the methyl substituent of octadecyltrimethylammonium chloride (OTAC) and DODMA have indicated that carbon atoms at these three positions were equally susceptible to biodegradation (Games et al. 1982; Sullivan 1983). Similarly, the use of uniformly labeled C_{18} chains indicated that each carbon position of the DODMA molecule was accessible to degradation and ultimately converted to CO_2 (Larson and Vashon 1983; Sullivan 1983). Furthermore, metabolic intermediates did not accumulate to appreciable levels and were also biodegraded to CO_2 (Sullivan 1983). Thus, DODMA might be expected to be

similarly degraded in soil over an extended period of time, with concurrent loss of the sorptive capacity of the modified soil.

The increased stability of HDTMA and DODMA in the B horizon soils and when bound to soil has been observed. The organic carbon and clay contents of the soil as well as their contribution to the CEC of the soil were proposed to influence the biostability of HDTMA and DODMA within the four different soils. The biostability of HDTMA and DODMA was assessed in a limited number of agricultural soils and thus it is difficult to extrapolate on the fate of these surfactants in aquifer materials. Therefore, further research on the stability of these surfactants in additional aquifer materials is advised.

SECTION V

BIOAVAILABILITY

A. INTRODUCTION

The influence of sorption on the biodegradation of organic contaminants is a poorly understood issue in bioremediation (Alexander 1991). Factors such as the compound's chemical structure, nature of the sorbent, the residence time of the sorbed compound and the desorption rate may influence the biodegradation of sorbed compounds. Additionally, the ability to directly utilize sorbed compounds may be a species-specific characteristic (Guerin and Boyd 1992). The contradictory results in the literature concerning whether or not a sorbed substrate is bioavailable may be due to the widely different substrate molecular structures and sorbent systems utilized, and the different models used to analyze the data.

We have investigated the bioavailability of naphthalene sorbed to a model sorbent, HDTMA-modified smectite, using both *P. putida* strain 17484 and *Alcaligenes* sp. strain NP-Alk. This work represented a continuation of research comparing the differential ability of these two strains to utilize naphthalene sorbed to soil (Guerin and Boyd 1992). In addition, we were interested in determining whether substrates sorbed to surfactant-modified soils would be available for microbial degradation. The results

from our work with the HDTMA-smectites are provided in a manuscript which comprises Appendix C.

The reference is:

Crocker, F.H., W.F. Guerin and S.A. Boyd. 199_. Bioavailability of naphthalene sorbed to cationic surfactant-modified smectite clay. Environ. Sci. Technol. (in press).

The results of an aging study in which lyophilized 50% HDTMA-smectite and naphthalene were equilibrated for approximately 6 months prior to determining the bioavailability of clay-sorbed naphthalene to strain 17484 are presented below. The methods used in this study are identical to those provided in the attached manuscript, except for the length of the equilibration as noted above.

B. RESULTS

Naphthalene mineralization curves in the presence of various concentrations of 50% HDTMA-smectite, equilibrated for 6 months are shown in Figure 7a. The mineralization of naphthalene by *P. putida* strain 17484 was rapid in all cases with sorption affecting naphthalene mineralization compared to the clay-free control (Figure 7a). The initial naphthalene mineralization rates in the presence of 50% HDTMA-smectite were greater than the rates predicted on the basis of the equilibrium aqueous phase naphthalene concentrations, as indicated by their position above the clay-free control line (Figure 7b). Furthermore, the extents of naphthalene mineralized (v_1/k values) in clay-containing

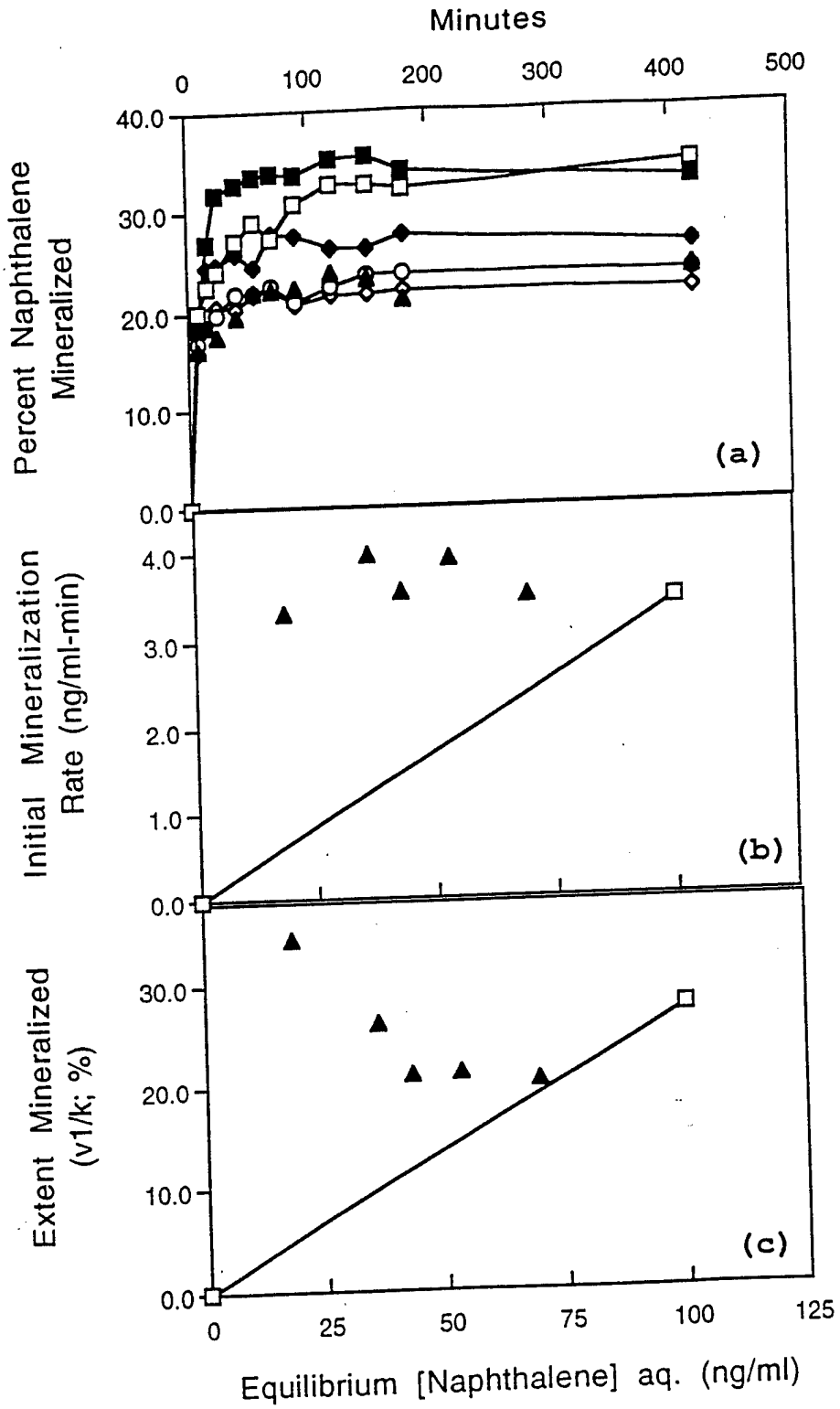


Figure 7. a: Naphthalene mineralization in clay-free (□) and 50% HDTMA-smectite slurries containing 2.5(◆), 5(o), 7.5(◊), 10(▲), or 25(■) mg clay/mL. b: Initial rates with 50% HDTMA-smectite(▲) or in a clay-free control(□) c: Extent of mineralization in a clay-free system or with 50% HDTMA-smectite. Upper x-axis for figure a, lower x-axis for figures b and c.

systems were generally higher than would be expected if sorbed naphthalene was unavailable and if desorption was slow relative to biodegradation (Figure 7c). However, a reduction in the extent of naphthalene mineralized compared to the clay-free control was noted in slurries containing 2.5 to 7.5 mg HDTMA-smectite/ml solution.

C. DISCUSSION

The long-term sorption of naphthalene to HDTMA-smectite did not significantly affect the availability of naphthalene to strain 17484. The high initial mineralization rates and extents of mineralization in the presence of the modified-clay were consistent with our earlier work with clay-sorbed naphthalene equilibrated for 24 hours. These studies suggest that *P. putida* strain 17484 is able to utilize a fraction of the surface-sorbed naphthalene and may promote the desorption of additional sorbed naphthalene by establishing steep intraparticle concentration gradients. The slightly reduced extents of naphthalene mineralized at the lower clay to solution ratios may indicate that volatile losses of naphthalene were high during the equilibration period and that sorption did not affect the extent of naphthalene mineralized. Furthermore, sorption did not limit naphthalene mineralization in slurries containing 10 and 25 mg/ml of modified-clay, since the extents of naphthalene mineralized were nearly equal to the value obtained in the clay-free control.

The stimulation of initial mineralization rates and extents of mineralization at high clay to solution ratios above the values observed in clay-free controls has been previosuly observed in HDTMA-smectite slurries (Crocker et al. in press) and in soil slurries (Guerin and Boyd 1992).

In contrast, naphthalene aging studies in soil slurries shifted the distribution of naphthalene toward the nonlabile sorbed phase. As expected, naphthalene mineralization rates decreased as the length of the equilibration period increased (Guerin and Boyd 1993). The apparent absence of an effect of aging on the bioavailability of clay-sorbed naphthalene may indicate that desorption rates from nonlabile sites within the non-aggregated modified-smectite are rapid or that nonlabile sites do not exist within the modified-clay structure. Evidence for the former hypothesis comes from observations of increased desorption rates of NOCs from HDTMA-modified soils and clays compared to natural soils (Benzing 1993). Thus, sorption of NOCs to cationic surfactant-modified soils is not expected to decrease the availability of the NOCs to microorganisms, since rapid sorbent desorption rates are characteristic of surfactant-modified soils and clays and some bacteria have the ability to utilize sorbed substrates.

SECTION VI

CONCLUSIONS

Coupling *in situ* immobilization of NOCs with their subsequent biological degradation is an attractive aquifer remediation technology. We investigated several aspects of this technology including QUAT binding to soils, the biostability and biocompatibility of the QUATs and the bioavailability of the immobilized NOC to degrading bacteria.

The chemical and biological stabilities of the HDTMA exchanged onto soils and clays was assessed. HDTMA soil complexes are chemically stable as long as the amount of HDTMA adsorption by hydrophobic bonding is limited, thus maximizing HDTMA adsoprtion to exchange sites. By lowering ionic strength and controlling the companion ion of HDTMA, HDTMA binding to exchange sites will be preferred and stability maximized. HDTMA is generally biologically stable in soils. The extent of mineralization is dependent on the location of exchange sites where HDTMA is bound and on environmental conditions. HDTMA biostability can be increased by: 1) binding to clay exchange sites, especially internal sites (e.g., smectite), 2) introduction to subsoils rather than surface soils and 3) maintaining saturated soil conditions.

The toxicity of HDTMA and DODMA to soil and aquifer bacterial populations was investigated. In addition, we assessed

the toxicity of these QUATs to a small number of bacteria isolated from these sites either prior to or after QUAT treatment. Although HDTMA is toxic to axenic cultures of bacteria, its toxicity is virtually eliminated by binding to clay minerals or soil clays. Reductions in bacterial diversity and density following soil treatment suggests an initial HDTMA toxicity. Gram-positive and spore-forming bacteria appear to be resistant to HDTMA and survive in HDTMA treated soils. These results indicate that a portion of the soil microbial population should survive the HDTMA treatment thereby retaining some degradative capability, and that repopulation of the treated zone should occur once HDTMA is bound to soil.

DODMA exhibited reduced toxicity compared to HDTMA, probably due to its dialkyl structure (Valko and Dubois 1945) and increased molecular weight (decreased water solubility) (James et al. 1987). This result along with other bactericidal studies on homologous series of different classes of QUATs suggest that alternate QUATs may be identified which are more compatible with indigenous soil and aquifer bacteria.

The treatment of soils and aquifer materials with HDTMA affected the activities of aerobic, heterotrophic bacteria. HDTMA treatment (at or near the CEC) increased the adaptation period prior to the mineralization of glucose, toluene, naphthalene and salicylate, and nearly inhibited phenanthrene and 2,4-D mineralization. This suggests that some important

contaminant degrading populations survive the initial HDTMA treatment and are able to rebound once the free HDTMA is exchanged onto soils or subsoils. However, other, perhaps less diverse, populations are virtually eliminated. Introduction of equivalent amounts of HDTMA prebound to soil eliminated inhibitory effects on mineralization of phenanthrene and 2,4-D, suggesting the likelihood that treated soils can be easily repopulated. The inhibitory affects were also reduced in materials with low CECs (such as subsoils or aquifer materials) which require a lower loading of HDTMA to achieve increased contaminant sorption. In these environments, microbial degradation of target contaminants would likely occur after an adaptation period following the initial surfactant treatment.

The long-term sorption of naphthalene to HDTMA-modified smectite did not significantly affect the bioavailability of naphthalene. Rapid NOC desorption rates are characteristic of surfactant-modified soils and clays (Benzing 1993; Burris and Antworth 1992). Therefore, sorption of NOCs to HDTMA-modified soils is not expected to decrease NOC bioavailability. Additionally, some bacteria are able to promote NOC desorption or directly access the sorbed contaminants (Guerin and Boyd 1992).

SECTION VII

RECOMMENDATIONS

The completed laboratory-scale studies have given us a greater understanding of QUAT binding to soils and subsoils, QUAT biostability, QUAT biocompatibility and NOC bioavailability. We recommend intermediate (pilot-scale) studies to further assess QUAT biocompatibility and biostability. Additionally, we recommend further research regarding methods of subsurface HDTMA introduction and its potential impact on soil physical properties including hydraulic conductivity. Premature field trials will almost certainly lead to a failure of the proposed technology.

Large QUATs such as HDTMA effectively displace native inorganic exchange cations forming stable organo-clay complexes and manifest low residual QUAT concentrations in solution. Thus, chemical stability is inherently high and can be further increased by controlling ionic strength and the type of companion ions.

We have shown that HDTMA is toxic to some bacteria and that treatment can substantially alter the indigenous population. However, we feel that once the added HDTMA is exchanged, there is the potential for repopulation of the treated zone by bacteria which survived the initial treatment and by those which are carried into the modified zone. Additional biocompatibility studies in a box model aquifer system which more closely represents actual field conditions are recommended to assess the

rate and extent of microbial repopulation and of contaminant biodegradation.

An evaluation of the biostability of the exchanged HDTMA in aquifer systems is also recommended. Our results suggest that pre-bound HDTMA is generally biologically stable in the agricultural soils tested. However, biostability results were somewhat inconsistent among replicates in a single exerpiment and even more so among different experiments. Therefore, the biostability of QUATs in aquifer systems where it is introduced as the free cation merits further study. Again, intermediate "box model aquifer" type studies are needed to address the biostability of the QUAT-clay complex under conditions simulating the field more closely.

SECTION VIII

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APPENDIX A

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Cation Exchange Chemistry of Hexadecyltrimethylammonium in a Subsoil Containing Vermiculite

Shihe Xu and Stephen A. Boyd*

ABSTRACT

Substituting native inorganic exchangeable cations of soil clays with cationic surfactants like hexadecyltrimethylammonium (HDTMA) greatly increases the sorption of organic contaminants, and hence reduces their transport potential. Both static and kinetic studies were conducted to probe the inorganic-HDTMA exchange processes in a subsoil containing vermiculite and to determine the chemical stability of the resultant HDTMA-soil complexes. The HDTMA generally had much higher affinity for the clay surface than inorganic cations native to soil and thus HDTMA saturation of soil resulted in a low residual HDTMA concentration in solution ($\approx 10^{-6}$ to 10^{-5} *M*). Conditions leading to flocculation of soil clays (e.g., high ionic strength or divalent exchangeable cations) resulted in inorganic cation entrapment in the interlayers of clays and decreased cation selectivity coefficients of inorganic-HDTMA exchange. Under these conditions, a portion of HDTMA was adsorbed via nonpolar interactions (hydrophobic bonding) resulting in a higher residual concentration of HDTMA in solution ($\approx 10^{-4}$ *M*) and a greater tendency for HDTMA desorption. The change in electrophoretic mobility of soil clays and turbidity of soil suspensions as HDTMA loading increased suggested that HDTMA, if adsorbed via cation exchange mechanism, caused clay aggregation and tended to distribute on the internal sites of the aggregates, leaving inorganic cations on external surfaces. The kinetic study revealed that inorganic-HDTMA exchange was fast on external surfaces but slow on internal sites of Al-hydroxy-interlayered vermiculites or flocculated vermiculites where the inorganic cation entrapment had been found. We concluded that the most stable HDTMA-soil complexes form at HDTMA loading of 0.6 to 0.7 cation-exchange capacity for nonsodic soils.

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SOIL CLAY MINERALS are generally ineffective sorbents for removing NOCs from water. In nature, the immobilization of aqueous phase NOCs results mainly from their partition interactions with soil organic matter (Chiou et al., 1979, 1983). Hence, low organic matter surface soils, subsoils, and aquifer materials have very limited capability for sorbing NOCs from water, and contaminant transport frequently occurs in these situations.

It has been shown that the sorptive capabilities of clay minerals, surface soils, and subsoils for organic contaminants can be improved substantially by replacing naturally occurring inorganic exchange cations with organic cations (Boyd et al., 1988a,b, 1991; Lee et al., 1989; Jaynes and Boyd, 1990, 1991). The organic cations studied most extensively are quaternary ammonium ions of the form $[RN(CH_3)_3]^+$, where R is an alkyl or aromatic hydrocarbon. Among the quaternary-ammonium compounds studied, HDTMA-modified clays or soils were among the most effective sorbents. After treating subsoils with HDTMA, sorption coefficients of several common organic groundwater contaminants such as benzene, toluene, and xylene increased by more than two orders of magnitude (Boyd et al., 1988a; Lee et al., 1989). In addition, HDTMA-modified clays can remove organic anions from water. Boyd et al. (1988c) studied PCP sorption by a series of organo-clays and found that HDTMA-modified clay was an effective sorbent for PCP

Abbreviations: HDTMA, hexadecyltrimethylammonium; CEC, cation-exchange capacity; NOC, nonpolar organic compound; PCP, pentachlorophenol; TBA, tetrabutylammonium; HIV, Al-hydroxy-interlayered vermiculite.

even at pH greater than its pK_a (4.7). Brixie and Boyd (1994) found that augmenting heavily contaminated soils with organo-clays substantially reduced the leaching of PCP even at pH 10.

The high sorption capability of modified clays and soils for organic pollutants makes it possible to use soil modification for in situ remediation (Boyd et al., 1988a, 1991; Burris and Antworth, 1990). The feasibility of underground injection of quaternary ammonium cations to create a sorptive zone that would intercept an advancing contaminant plume and immobilize organic contaminants therein has been demonstrated (Burris and Antworth, 1992). This, coupled with subsequent biodegradation of the immobilized contaminants could be used as a comprehensive soil restoration technology (Burris and Antworth, 1990; Nye et al., 1994). Two aspects of clay or soil modification that have not been well studied are the adsorption of quaternary ammonium cations on soil clays and the stability of the resultant organo-clay complexes.

The objectives of this study were to (i) evaluate kinetic and equilibrium aspects of HDTMA adsorption and desorption, (ii) determine how they are affected by various soil factors such as soil clay type and the type and concentration of electrolytes in soil solution, and (iii) relate adsorptive mechanisms to the chemical stability of the organo-clay complexes.

MATERIALS AND METHODS

Soil Samples

Two subsoil samples from Kellogg Biological Station, Hickory Corners, MI (Horizons Bt and C from Oshtemo soil, a coarse-loamy, mixed, mesic Typic Hapludalf) were used in this study (Table 1). These materials were air dried and passed through a 1-mm sieve. Both Na- and Ca-saturated soils were made by repeated washing of the soils with 1 *M* NaCl or 0.1 *M* $CaCl_2$ solutions. The Na-saturated or Ca-saturated soils were characterized for clay and organic C content, CEC, and the amount of exchangeable cations extracted by TBA and by x-ray diffraction patterns. Organic C content was determined commercially by combustion (Huffman Lab., Inc., Golden, CO). Cation-exchange capacity was determined using the NH_4OAc method (Rhoades, 1982), except we used both Na and Ca as index cations and $BaCl_2$ solution as the extracting reagent. The amount of exchangeable cation extractable by TBA was determined in the same way as the CEC except that Na was the index cation and 0.1 *M* TBA chloride the extracting reagent. In addition, soil was Cs-saturated and heated at 105°C overnight to collapse vermiculites before Na saturation. The CEC of the Cs-treated soils was then determined using the method described above. The x-ray diffraction patterns of oriented Ca- and K-saturated soil clays were obtained for air-dried and 105°C oven-dried samples using a x-ray diffractometer (Philips APD3720, Philips Electronic Instrument Inc., Mahwah, NJ).

Adsorption of HDTMA

Adsorption of HDTMA to both soil samples in different initial NaCl and $CaCl_2$ solutions was examined to determine the cation selectivity coefficients of Na (or Ca)–HDTMA exchange and the influence of clay dispersion on inorganic cation–HDTMA exchange. Stock solutions of 0.0225 *M* HDTMA chloride were made by mixing methyl-^{14}C-labeled HDTMA (as HDTMA-I from American Radiolabeled Chemicals, St. Louis, MO, radiochemical purity >97%, specific activity 2.2×10^{11} Bq $mmol^{-1}$) with a solution of unlabeled HDTMA chloride to yield HDTMA/^{14}C-HDTMA ratios between 1000:1 and 10000:1. The amount of soil having ≈0.09 mmol (+) exchangeable cation (e.g., 2.4 g for Oshtemo B) was weighed into 25-mL Corex centrifuge tubes (Thomas Scientific, Swedesboro, NJ) and washed four times with 25 mL of either 1 m*M* NaCl or 5 m*M* NaCl or 1 m*M* $CaCl_2$ to remove the extra salts left from soil saturation by Na or Ca. The two NaCl concentrations (1 and 5 m*M*) were chosen to achieve highly dispersed and flocculated clay suspensions, respectively, based on our previous observation. After mixing thoroughly, the samples were centrifuged and most of the supernatant (22–24 mL) removed. Identical volumes of the NaCl or $CaCl_2$ solutions were then added to the tubes. After the fourth wash, the appropriate volume of standardized NaCl or $CaCl_2$ solutions and H_2O was pipetted into the tube and mixed well to redisperse the soil. Volumes of stock HDTMA equivalent to 0.1 to 2.5 times the CEC were injected into each tube and mixed. The tubes were shaken for 3 d, vortexed, and then allowed to stand for 2 h before the upper ≈5 mL of suspension was removed for turbidity measurement (described below). The remaining solution and soil were separated by centrifugation (9.68 kg for 20 min). The ^{14}C activity in the stock solution and in the supernatant samples was determined by liquid scintillation counting (Tri-Carb 1500 Liquid Scintillation Analyzer, Packard Instrument Co., Downers Grove, IL). The Na or Ca liberated was measured with flame emission and atomic absorption spectrophotometry (Perkin-Elmer, 1100B, Norwalk, CT), respectively. Total HDTMA adsorption was determined by the difference between ^{14}C activity in the initial solution and in the supernatant obtained after equilibrating the soil. This method was validated by soil combustion to convert adsorbed ^{14}C-HDTMA into $^{14}CO_2$ (Harvey Biological Oxidizer, R.J. Harvey Inst. Co., Hillsdale, NJ), which was trapped in alkaline scintillation cocktail. A regression of these two independent measurements for 12 samples yielded the following equation: [HDTMA] by combustion = 1.03 [HDTMA] by difference + 0.05 (R^2 = 0.988).

Cation selectivity coefficients of Na–HDTMA ($^cK_{Na\text{-}HDTMA}$) and Ca–HDTMA exchange ($^cK_{Ca\text{-}HDTMA}$) were calculated using the following equations:

$$^cK_{Na\text{-}HDTMA} = (a_{Na}/a_{HDTMA}/N_{Na}) \quad [1]$$

$$^cK_{Ca\text{-}HDTMA} = (a_{Ca}/a^2_{HDTMA})(N^2_{HDTMA}/N_{Ca}) \quad [2]$$

where a is the activity of cations in solution and N is the mole fraction of a cation on the surface.

Table 1. Selected properties of Oshtemo subsoil.

	Oshtemo B	Oshtemo C
pH	5.8	7.1
Clay content, g kg^{-1}	60	40
Organic C content, g kg^{-1}	1	<1
CEC, $mmol_c$ kg^{-1}	38	12
CEC_{TBA}, $mmol_c$ kg^{-1}	30.4	8
CEC_{Cs}, $mmol_c$ kg^{-1}	25.1	8.3

Microelectrophoresis and Soil Clay Dispersion

Electrophoretic mobility and turbidity of supernatant samples from the HDTMA adsorption experiment were measured to monitor changes in surface charge and associated clay dispersion as HDTMA loading increased. Light absorption at 375 nm by the upper 5 mL of supernatant was measured as an

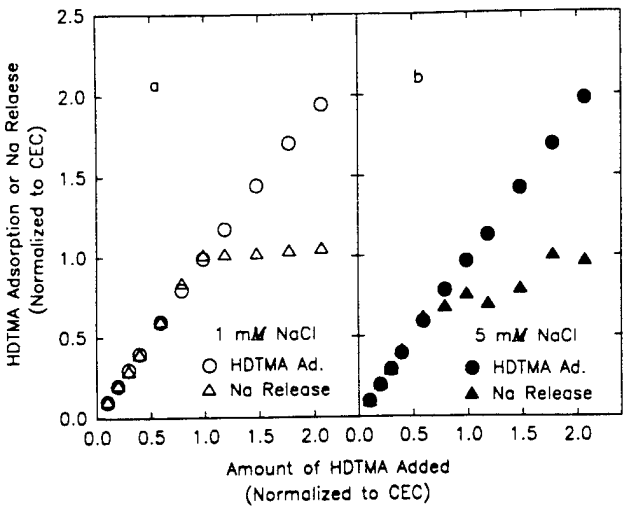

Fig. 1. Comparison of the amount of hexadecyltrimethylammonium (HDTMA) adsorbed to the amount of Na liberated in Na-saturated Oshtemo B soil at two initial NaCl concentrations (a and b). All of the variables (HDTMA adsorption, Na release, and the amount of HDTMA added to soil suspension) are normalized to the soil CEC (38 mmol$_c$ kg^{-1}).

index of dispersion using a spectrophotometer (Spectronic 20, Bausch and Lomb Inc., Rochester, NY). The soil–water suspension was then centrifuged, and supernatant samples for ^{14}C activity and Na and Ca release were taken. Most of the remaining supernatant was transferred to a Teflon (Thomas Scientific) electrophoretic cell. The soil pellet was redispersed in the residual ($\approx$2 mL) supernatant and $\approx$0.1 mL of the soil suspension was added to the cell to make a dilute ($\approx$0.01%) suspension. The electrophoretic mobility of 25 to 50 particles was recorded for each sample using a microelectrophoretic apparatus (Zeta Meter 3.1, Zeta Meter Inc., New York).

Desorption of HDTMA

Desorption of HDTMA was measured to determine the reversibility of inorganic cation–HDTMA cation-exchange reaction. Supernatant from the adsorption experiments was quantitatively removed from the sample tubes. An equal volume of aqueous NaCl, at a concentration equal to that in the adsorption experiment, was added into the tubes, and the soil was redispersed by vortex, shaken for 3 d, and centrifuged. The ^{14}C activity and Na concentration in the supernatant were measured as described above. The HDTMA desorbed was calculated by subtracting the ^{14}C activity arising from the residual solution that could not be completely removed in the previous step. This procedure was repeated four times.

Kinetics of Na–HDTMA Exchange

Kinetics of HDTMA adsorption and Na release in both the untreated and Cs-treated soils were determined in 1 m*M* and 5 m*M* NaCl to evaluate the influence of exchange sites and ionic strength on the kinetics of Na–HDTMA exchange. The Na-saturated soils (Cs-treated or untreated) were dispersed in NaCl solution of known concentration. A volume of HDTMA stock solution containing ^{14}C-labeled HDTMA corresponding to 1.5 times the CEC of the soil was added to the soil suspension. After certain time intervals (from 0.5–120 h), the soil was centrifuged and the Na concentration and ^{14}C activity in supernatant were determined as described above.

RESULTS

Soil Characterization

Both subsoil samples have low clay and organic matter contents (Table 1). The CEC of the soils were low, and a portion of the exchange sites was inaccessible to TBA (Table 1). Cesium-saturation plus oven drying reduced the CEC of the soil (Table 1). X-ray diffraction of Ca-saturated soil clay showed a broad peak at 1.45 nm and weak peaks at 1.0 and 0.72 nm. Potassium saturation shifted the 1.45-nm peak to 1.42 nm and reduced its peak intensity but increased the intensity of the 1.0-nm peak.

HDTMA Adsorption in Na-Saturated Soil

Hexadecyltrimethylammonium adsorption in Na-saturated soils was affected by the initial NaCl solution concentrations. When the concentration was low (1 m*M*), HDTMA adsorption resulted in equivalent Na release at HDTMA loading levels less than or equal to the CEC of the soil (Fig. 1a). The total adsorption of HDTMA exceeded the CEC at higher HDTMA loadings. In the more concentrated NaCl solution (5 m*M*), the overall adsorption of HDTMA was similar, but the adsorption of HDTMA resulted in stoichiometric release of Na from exchange sites only when the HDTMA loading was $\leq$0.6 CEC (Fig. 1b), after which HDTMA adsorption exceeded Na release.

Whether the HDTMA adsorption resulted in equivalent Na release determined the residual concentration of HDTMA in equilibrium solution for a given degree of exchange site saturation (Fig. 2a). The equilibrium concentration of HDTMA was $<10^{-5}$ *M* for HDTMA loading up to 1 CEC in 1 m*M* NaCl but only up to 0.6 CEC in 5 m*M* NaCl. In addition, the Na–HDTMA exchange isotherm (Fig. 2a) exhibited an unusual feature in that the equilibrium concentration of HDTMA was lowest at intermediate loading levels.

The influence of the initial NaCl concentrations on Na–HDTMA exchange was also reflected in the cation selectivity coefficients, a measure of the surface affinity of HDTMA relative to inorganic cations. Logarithm of cation selectivity coefficients for Na replaced by HDTMA (log $^cK_{Na\text{-}HDTMA}$) ranged from 1 to 4.6 (Fig. 2b). The individual cation selectivity coefficients depended on the HDTMA loading level and the initial concentration of background electrolytes. In dilute NaCl solution, log $^cK_{Na\text{-}HDTMA}$ started higher and increased with HDTMA loading up to 0.6 CEC. Between 0.6 and 0.8 CEC, log $^cK_{Na\text{-}HDTMA}$ decreased.

HDTMA Adsorption in Ca-Saturated Soil

Hexadecyltrimethylammonium adsorption in Ca-saturated soil was nearly linearly related to the logarithm of the HDTMA equilibrium concentration in solution and did not depend on the initial $CaCl_2$ concentration (Fig. 3). Calcium–HDTMA exchange in $CaCl_2$ resembled Na–HDTMA exchange in 5 m*M* NaCl solution in one aspect. Namely, the loading level corresponding to

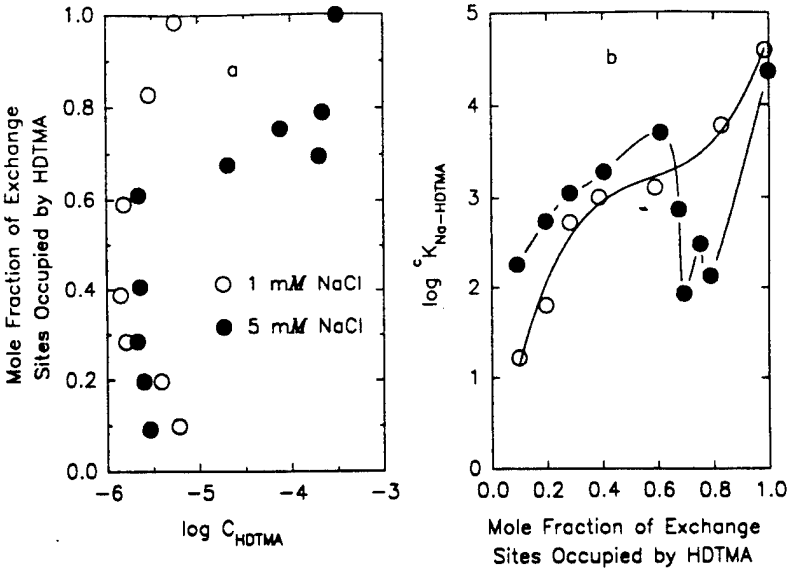

Fig. 2. (a) Sodium-hexadecyltrimethylammonium (HDTMA) exchange isotherms for Na-saturated Oshtemo B at two initial NaCl concentrations and (b) the dependence of cation selectivity coefficients ($^cK_{Na-HDTMA}$) on the HDTMA loading level.

the decrease in log $^cK_{Ca-HDTMA}$ was ≈0.6 CEC for both soils.

Desorption of HDTMA in NaCl Solutions

Selectivity coefficients calculated from desorption were comparable to those obtained from adsorption (Fig. 4). Generally speaking, the desorbability of the adsorbed HDTMA on exchange sites depended on the HDTMA loading level (Fig. 4). When HDTMA loading levels were <0.8 CEC, selectivity coefficients calculated from desorption in the dilute (1 m*M*) NaCl solution were almost identical to the selectivity coefficients calculated from adsorption. When HDTMA loading levels were >0.8 CEC, we observed higher cation selectivity coefficients in desorption than those in adsorption, a phenomenon referred to as *desorption hysteresis*. Similarly, we observed no hysteresis at HDTMA loading levels <0.6 CEC in 5 m*M* NaCl, but desorption hysteresis was observed at higher loading levels.

Electrophoretic Mobility of HDTMA Partially Exchanged Soil and Turbidity of Soil Suspension

Regardless of the initial NaCl concentration, the mixed Na-HDTMA soil clay had a S-shaped electrophoretic mobility vs. HDTMA loading curve (Fig. 5a). At low loading levels, the electrophoretic mobility of the clay was negative and remained constant as HDTMA loading increased. A sharp transition from negative to positive mobility occurred as HDTMA loading increased to a critical level corresponding to ≈1 CEC for soil in the

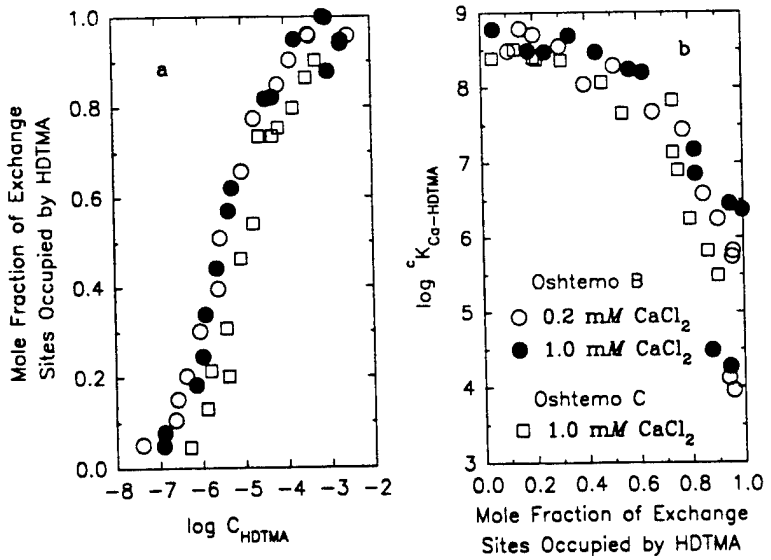

Fig. 3. (a) Calcium-hexadecyltrimethylammonium (HDTMA) exchange isotherms for Ca-saturated Oshtemo B and C soils at two initial $CaCl_2$ concentrations and (b) the dependence of cation selectivity coefficients ($^cK_{Ca-HDTMA}$) on HDTMA loading level.

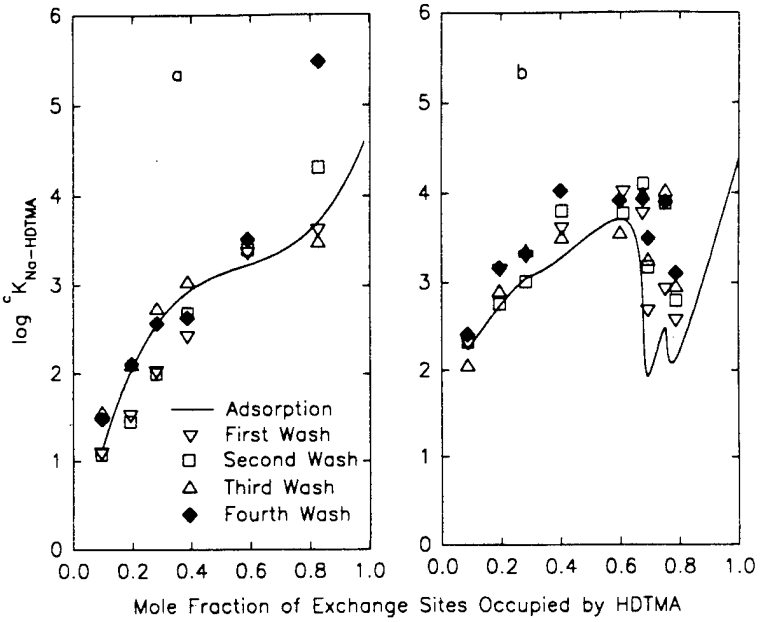

Fig. 4. (a) Comparison between cation selectivity coefficients of Na-hexadecyltrimethylammonium (HDTMA) exchange on Oshtemo B obtained from adsorption (Na-HDTMA) with (b) those from desorption (HDTMA-Na).

dilute (1 m*M*) NaCl solution in which stoichiometric cation release was observed (Fig. 1). However, this critical HDTMA loading level was <1 CEC in 5 m*M* NaCl where HDTMA adsorption did not result in stoichiometric replacement of Na at HDTMA loadings >0.6 CEC.

Unlike the electrophoretic mobility, turbidity of soil clay suspension was very sensitive to HDTMA loading in 1 m*M* NaCl solution. As Na was exchanged by HDTMA, the turbidity as represented by optical density decreased at low loading level and reached a minimum near 1 CEC (Fig. 5). Further increases in HDTMA loading beyond this point resulted in increased turbidity. Turbidity of soil suspensions in 5 m*M* NaCl was very low at HDTMA loadings ≤1 CEC and increased with HDTMA loading thereafter.

Kinetics of HDTMA Adsorption and Na Release

Hexadecyltrimethylammonium adsorption was faster than Na release regardless of the ionic strength (Fig. 6). The difference was especially large when the soil clay was flocculated before HDTMA was applied to the soil suspension (e.g., in 5 m*M* NaCl). Also, the Na-release rate was fastest initially but became slower as the HDTMA loading increased. Increasing ionic strength decreased Na release rates for soil (Fig. 6). Release rates of exchangeable Na were independent of ionic strength in

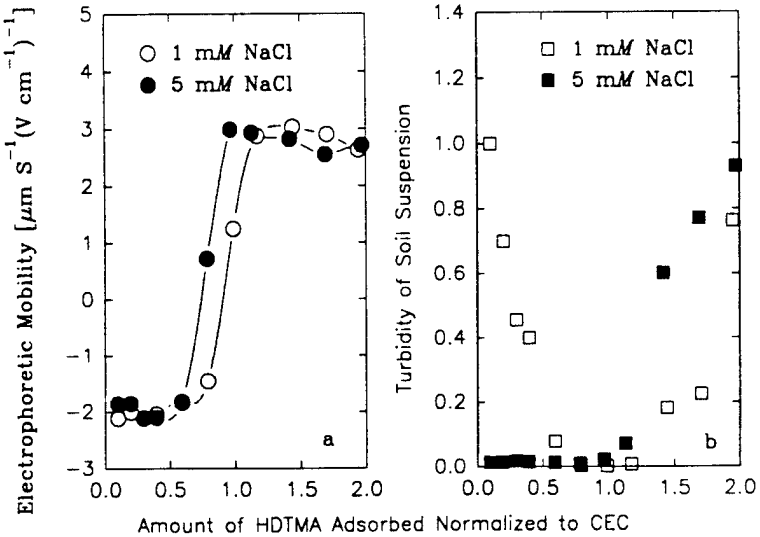

Fig. 5. (a) Electrophoretic mobility of soil clasy in Oshtemo B soil and (b) turbidity of soil suspension as affected by hexadecyltrimethylammonium loading.

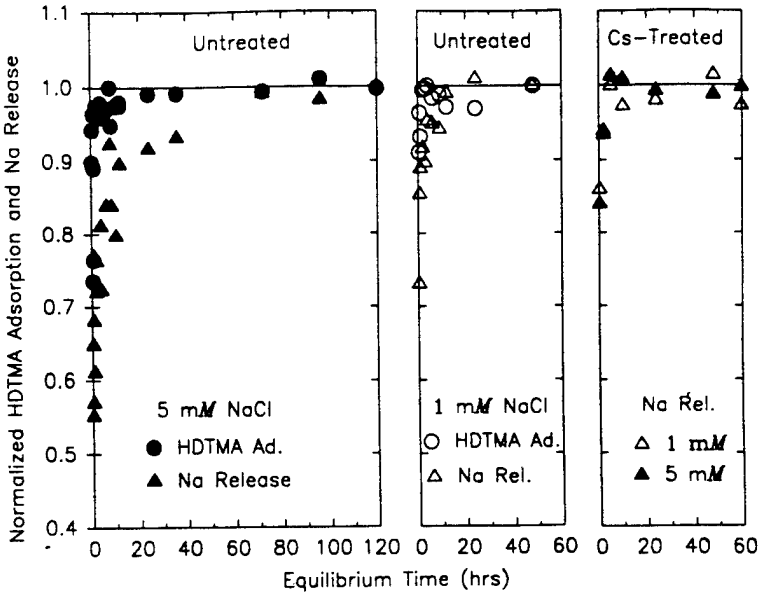

Fig. 6. Kinetics of hexadecyltrimethylammonium adsorption and Na release on Na-saturated untreated Oshtemo B soil and on Na-saturated Cs-treated Oshtemo B soil as affected by initial NaCl concentrations.

soil treated with Cs to collapse the interlayers of vermiculite (Fig. 6).

DISCUSSION

Soil Clay Characterization

The Oshtemo soil contains vermiculite, Al-hydroxy-interlayered vermiculite (HIV), illite, and kaolinite. The broad 1.45-nm peaks for both samples actually consisted of 1.49- and 1.42-nm peaks, corresponding to Ca-vermiculite and HIV, respectively. Potassium treatment collapsed the vermiculite, resulting in disappearance of the 1.49-nm peaks. This revealed a 1.42-nm peak and increased the intensity of the 1.0-nm peak. Peaks of 0.72 and 1.0 nm of Ca-saturated soil clay confirmed the existence of kaolinite and illite (Lietzke and Mortland, 1973).

The CEC of the soil can be expressed in the following component terms:

$$CEC = CEC_{ext} + CEC_{HIV} + CEC_V + CEC_{org} \quad [3]$$

where CEC_{ext} is total amount of external exchange sites per unit mass of soil, CEC_{HIV} refers to exchange sites located inside the micropores of HIV, CEC_V refers to exchange sites located in the interlayers of vermiculite, and CEC_{org} is the contribution of CEC from organic matter. Organic matter contribution to total CEC was small (maximum 7% for Oshtemo Bt and <10% for Oshtemo C based on their low organic matter contents and typical CEC of organic matter [Helling et al., 1964]) and was omitted from the above equation. The other terms can be measured directly or calculated from the values shown in Table 1. Tetrabutylammonium is a bulky cation (diameter >0.5 nm) that is excluded from exchange sites inside the micropores of HIV (≈0.46 nm), thus $CEC - CEC_{TBA}$ represents the amount of exchange sites inside HIV (CEC_{HIV}). Cesium saturation plus overnight heating collapsed vermiculite interlayers, and thus, the amount of Na exchanged in a Cs-treated soil, i.e., CEC_{Cs}, represents the exchange sites left after eliminating the contribution from vermiculite interlayers, hence $CEC - CEC_{Cs} = CEC_V$. The calculated results show that for the Oshtemo B and C soils, HIV contributed 20 and 33% of the CEC, respectively, and vermiculite interlayers contributed 34 and 31% of the CEC, respectively.

Static Study: HDTMA Adsorption

Organic cations like HDTMA can be adsorbed through two mechanisms: cation exchange and nonpolar interactions of the C-16 alkyl chains of HDTMA (hydrophobic bonding). To examine the cation-exchange reaction separately when both adsorption mechanisms were operative, we assumed that Na liberated by HDTMA represented the amount of HDTMA adsorbed on exchange sites. This assumption is justified as follows. First, H^+ competition for exchange sites could increase the Na concentration in solution. However, the high equilibrium Na concentration (1.2–14 m*M*) prevented this as indicated by the pH of the NaCl–HDTMA solution, which remained relatively constant during the exchange processes (pH 5.2–5.8, data not shown). Second, Na exclusion due to positive charge development on the clay surface resulting from HDTMA adsorbed via hydrophobic bonding could cause an increase in measured Na concentrations in the bulk solution. However, this effect was arguably small at HDTMA loadings <1.5 CEC. The extent to which the measured concentration of Na will be higher than its true concentration (i.e., the extent of cation exclusion) ΔC_i (mol L^{-1}) can be expressed by Eq. [4], developed from the equation of Chan et al. (1984):

$$\Delta C_i = 2\,(C_i/V_T)A/\kappa)[1 - \exp(-Z_iF\psi_d/2RT)]$$
$$\psi_d \geq 0 \quad [4]$$

where C_i and V_T are measured concentration of ion i (e.g., Na) and total volume of solution, A is area of surface exposed to solution, ψ_d is the electrostatic potential at the outer Helmotz plane, κ is the Debye-Hückel parameter, Z_i is the valency of ion i, and F, R, and T are Faraday's constant, the ideal gas constant, and temperature (in K), respectively. Under our experimental conditions (V_T = 25 mL, T = 298 K), therefore

$$\Delta C_i = 7.716 \times 10^{-7} C_i^{1/2} A [1 - \exp(-19.47\psi_d)] \quad \psi_d \geq 0 \quad [5]$$

In our experimental system, the two factors directly affecting Na exclusion, ψ_d and A, are both small. When HDTMA loading was <1 CEC of the soil in 1 m*M* NaCl solution or <0.6 CEC in 5 m*M* NaCl solution, ψ_d are either negative or zero as indicated by the negative or zero electrophoretic mobility (Fig. 5) and no significant Na exclusion is expected. As the HDTMA loading increased, the surface charge became positive (Fig. 5). However, the clay particles were aggregated at HDTMA loadings of 0.6 to 1.5 CEC, as indicated by the low turbidity of the soil suspension (Fig. 5), and the area of external surface responsible for cation exclusion (i.e., A in Eq. [5]) was, therefore, expected to be small. Even when we assume A = 70 000 m^2 kg^{-1} (equivalent to pure illite) for soil clays in the flocculated state, in a 5 m*M* NaCl solution, ΔC_{Na} will be only 0.19 m*M* according to Eq. [5]. This will translate to 5.3% relative error in Na release measurement. Similarly, the relative errors will be 2.4 and 7.5% in 1 and 10 m*M* NaCl, respectively.

The ability to measure the HDTMA exchange reaction by monitoring Na release allows us to examine the influence of ionic strength and type of inorganic cation on inorganic–HDTMA exchange. One obvious effect of ionic strength on Na–HDTMA exchange is to change the critical loading level at which the stoichiometric Na release no longer occurs concomitantly with HDTMA adsorption. In dilute NaCl solution, HDTMA adsorption resulted in equivalent Na release at HDTMA loading ≤ 1 CEC, suggesting cation exchange as the only operative adsorption mechanism. The fact that HDTMA adsorbed via hydrophobic bonding became significant only after all the exchangeable sites were saturated with HDTMA (Fig. 1a), suggests that cation exchange results in a more stable HDTMA surface complexes than hydrophobic bonding. In more concentrated NaCl solution or in $CaCl_2$ solution, inorganic cations became more difficult to exchange after a certain mole fraction (≈0.6) of exchange sites were occupied by HDTMA. This was indicated by substantial increase in the aqueous HDTMA concentration (Fig. 2) and a decrease in log ${}^cK_{Na\text{-}HDTMA}$ or log ${}^cK_{Ca\text{-}HDTMA}$ (Fig. 2 and 3). Under these conditions, hydrophobic bonding was operative before all the inorganic cations were replaced, implying that it can be a competitive adsorption mechanism at higher ionic strength or in divalent cation solutions.

The above results are consistent with the data from previous studies on organic cation adsorption on clay minerals (Theng et al., 1967; Vansant and Peeters, 1978) with one exception. Consistent with our results, Theng et al. (1967) observed that log ${}^cK_{Na\text{-}org}$ were initially low and increased with organic cation loading level up to ≈0.7 CEC and then decreased independent of ionic strength, and that log ${}^cK_{Ca\text{-}org}$ was initially high and decreased with loading level. However, we only observed a decrease in log ${}^cK_{Na\text{-}org}$ (at high organic cation loadings) in NaCl solutions concentrated enough to cause flocculation of soil clays.

The drastic decrease in the selectivity coefficient of HDTMA at high loading levels is due to a phenomenon referred to as cation entrapment, where the species being exchanged (e.g., Na) becomes inaccessible. Cation entrapment has been observed in inorganic systems when vermiculite is brought to contact with K, Rb, or Cs solutions, especially at high concentrations (Sridhar and Jackson, 1973; Klobe and Gast, 1970). The entrapment is caused by rapid edge collapse but charge heterogeneity may also play a role (Sawhney, 1969). For organic–inorganic exchange, cation entrapment has also been observed previously. Greenland and Quirk (1962) reported that hexadecylpyridinium could entrap up to 25% of the Na in Wyoming montmorillonite.

In a mixed (organic–inorganic) system, several factors may influence cation entrapment, including type of minerals, the type of exchangeable inorganic cation initially on the exchange sites, the type and concentration of inorganic cation initially in bulk solution, and the size of the organic cations. Tetrapropylammonium can replace >50% of the Ca from Wyoming montmorillonite but only ≈10% from Libby vermiculite (McBride and Mortland, 1973). Using large quaternary ammonium cations, McAtee (1959) observed an almost stoichiometric replacement of Na in Montmorillonite. Under the same conditions, considerably less Ca was exchanged. Our observation that cation entrapment occurred in dilute $CaCl_2$ solution but not in dilute NaCl solution (1 m*M*) confirmed the cation type effects. Our observation that Na entrapment in NaCl solutions occurred only in high NaCl concentration (5 m*M*) suggests ionic strength as another important factor influencing cation entrapment.

Two different explanations have been used for cation entrapment. Cation entrapment was first described as a *cover-up effect* (Hendricks, 1941) where the organic species may occupy or cover an area exceeding the area per exchange site thus obscuring an adjacent exchange cation. Cation entrapment has also been ascribed to interlayer contraction (McBride and Mortland, 1973) which limits access of organic cations to the interlayers. However, Na entrapment in montmorillonite or vermiculite when large organic cations like HDTMA are present cannot be accounted for by either the cover-up effect or interlayer contraction because the interlayer arrangement of organic cations changes (e.g., to a vertical orientation relative to the clay sheet) in response to the surface charge density of the clay (Lagaly, 1982) and the basal spacings of such organic-montmorillonite or vermiculite clays are even larger than pure Na or Ca clays (Jaynes and Boyd, 1991), i.e., the layers are not contracted. We propose that Na or Ca entrapment by HDTMA in the soil studied occurs by a different mechanism. This results from the restricted

access of HDTMA to the exchange sites, which is influenced by both ionic strength and exchange cation type. When Na-saturated soil clay was highly dispersed in 1 m*M* NaCl, HDTMA could access all exchange sites and replace all the Na. For the Na soil in 5 m*M* NaCl, or Ca soil in $CaCl_2$ solution, the clay became flocculated (e.g., particles formed face-to-face aggregates) before HDTMA was added to the soil suspension. The access of HDTMA to exchange sites was restricted to those on the external surfaces and those near the edges. Replacement of interlayer Na or Ca by HDTMA near the edges created a hexadecyl-chain barrier along these edges. The hydrophobic nature of this barrier makes it difficult for the hydrated Na or Ca to diffuse out from the interlayer. As a result, Na and Ca becomes difficult to replace after reaching a certain loading level of HDTMA. We refer to this as the *barrier effect.*

The dependence of log ${}^cK_{Na\text{-}HDTMA}$ and log ${}^cK_{Ca\text{-}HDTMA}$ on HDTMA loading and initial NaCl solutions agrees with the above argument. It is known that the much higher affinity of organic cations than inorganic cations on the clay surface is partly due to lateral interactions of adsorbed organic cations (cooperative adsorption) (Theng, 1974; Rosen, 1989). Cooperative adsorption stabilizes the organo-clay complex because the hydrophobic chains of organic cations like HDTMA associate by nonpolar interactions when they are in close proximity on surface. The greater access of HDTMA to exchange sites in dilute NaCl solution may result in a more random distribution of HDTMA and Na on the clay surface. Hence, the likelihood of one adsorbed HDTMA being in close proximity to another HDTMA on the surface, or of localized patches of HDTMA adsorbed together, is less than that in more concentrated NaCl solution or in $CaCl_2$ solutions where HDTMA is concentrated along the edges of clay particles. This leads to higher residual concentrations of aqueous HDTMA and smaller log ${}^cK_{Na\text{-}HDTMA}$ at low loading levels ($\leq$0.2 CEC) in 1 m*M* NaCl solution than in 5 m*M* NaCl solution. Differences in the interlayer arrangement of HDTMA in 1 and 5 m*M* NaCl should decrease as the HDTMA loading level increases, and hence, the difference in log ${}^cK_{Na\text{-}HDTMA}$ values under those conditions becomes less at intermediate loading levels ($\approx$0.2–0.6 CEC) than that at loadings <0.2 CEC. The drastic decrease of log ${}^cK_{Na\text{-}HDTMA}$ at HDTMA loadings >0.6 CEC in 5 m*M* NaCl reflects the barrier effect described above, rather than an equilibrium phenomenon. The independence of Ca–HDTMA exchange isotherms on initial $CaCl_2$ solution concentrations and lack of increases in cation selectivity coefficients at low HDTMA loading reflects the fact that access of HDTMA to the exchange sites on Ca clay is more restricted than that of Na clay.

Alternatively, the dependence of log ${}^cK_{Na\text{-}HDTMA}$ on loading level could be due to multi-type exchange sites because the soil contains a mixture of clays (Table 1) and both Na and HDTMA may have higher affinities for some sites than others. However, the data shown in Fig. 2 do not support this interpretation. In dilute NaCl background, HDTMA should be initially adsorbed on high affinity sites and hence the observed log ${}^cK_{Na\text{-}HDTMA}$ should be high at low HDTMA loading levels. As the HDTMA loading level increases, HDTMA would saturate the high affinity sites and then occupy only lower affinity sites, resulting in a decrease in the log ${}^cK_{Na\text{-}HDTMA}$ rather than the observed increase. Our data imply that if there is any change in the affinity of sites, the effect is overridden by cooperative adsorption.

The cation arrangement we propose for 5 m*M* NaCl or $CaCl_2$ solutions that results in cation entrapment is consistent with the concept of demixing into domains proposed previously for organic cation adsorption by vermiculite (McBride and Mortland, 1973). However, it should be emphasized that cation entrapment described herein is caused by steric hindrance and thus is a nonequilibrium phenomenon in contrast to demixing into layers or domains which are equilibrium phenomena (Theng et al., 1967; Barrer and Brummer, 1963; McBride and Mortland, 1973). The random distribution of HDTMA in the interlayers in 1 m*M* NaCl can be considered as a metastable state, formed by the sudden exposure of all exchange sites to the HDTMA, because the rearrangement of HDTMA into the most stable configuration in the interlayer region is retarded due to the low mobility of HDTMA in the interlayers.

Static Study: Electrophoretic Mobility of Soil Clay and Turbidity of Soil Suspension

Another aspect of cation arrangement in HDTMA-exchanged soils concerns the distribution of HDTMA and Na between the interlayers and external surfaces of soil clays. In a mixed Na–Ca–montmorillonite system, Bar-On et al. (1970) reported that the negative electrophoretic mobility increased dramatically as the Ca–montmorillonite is mixed with even small amounts of Na–montmorillonite because Na is less effective at screening negative surface charge, leading to more negative electrophoretic mobility. This rapid change of electrophoretic mobility continues until exchangeable Na reaches 30% of the CEC of the clay although the quasicrystal structure of Ca–montmorillonite does not change. Thereafter no further change in electrophoretic mobility is observed, but quasicrystal structures start to break up. Because the electrophoretic mobility is only sensitive to external surface potential, the drastic change in electrophoretic mobility with little change in quasicrystal structure is attributed to demixing of cations, i.e., Na occupying the external sites, whereas Ca remains in interlayers which maintains the quasicrystal structure.

Our electrophoretic mobility and turbidity data also support a demixing process where Na occupies the external sites and HDTMA is in the interlayers. This promotes face-to-face association of clay particles due to the mutual attraction of hydrophobic tails of organic cations (Theng, 1974). This is similar to the mixed Na–Ca–montmorillonite described by Bar-On et al. (1970). In dilute NaCl solution, addition of HDTMA resulted in a sharp decrease in the turbidity of the soil suspension, indicating the formation of aggregates. However, HDTMA adsorption did not change the electrophoretic mobility until it reached to relative high loading levels ($\approx$0.7 CEC),

suggesting that HDTMA did not occupy the external surface sites of the aggregates. In other words, HDTMA loading up to ≈0.5 CEC resulted in decreased clay dispersion and decreased external surface area but no change in the composition of exchange cations on external surfaces as indicated by constant electrophoretic mobility (Fig. 5). This demixing continues until the aggregates are so large that no more face-to-face association occurs and the turbidity of the soil suspension reaches its minimum. A small HDTMA addition after this point results in replacement of Na on the external surfaces and thus a sharp change of electrophoretic mobility towards zero or even a positive value if a small amount of HDTMA is adsorbed via hydrophobic bonding. This occurred at HDTMA loading near 1 CEC when no cation entrapment was involved but <1 CEC when cation entrapment occurred.

Static Study: HDTMA Desorption

Most existing data on inorganic–organic cation-exchange reactions on clays have been obtained for the forward reaction (inorganic replaced by organic). Use of those data to represent the thermodynamic exchange equilibrium, assumes that the exchange reaction involving organic cations is reversible (Theng, 1974). As Fripiat et al. (1965) have demonstrated, this type of macroscopic reversibility is seldom realized in montmorillonite and vermiculite systems primarily because the interlayer separation in a crystal varies during the course of the reaction. An exchange resulting in d-spacing contraction (e.g., Na–K) may cause desorption hysteresis. Our desorption studies (Fig. 4) show that the extent of HDTMA loading on the exchange sites affects the reversibility of Na–HDTMA exchange. The large desorption hysteresis at high loading levels (>0.8 CEC) in 1 m*M* NaCl solution may be caused by cooperative adsorption effects because there is no d-spacing contraction.

In addition, large desorption hysteresis was observed when cation entrapment occurred in the adsorption step. As stated earlier, the drastic decrease in log ${}^{c}K_{Na\text{-}HDTMA}$ or log ${}^{c}K_{Ca\text{-}HDTMA}$ at high HDTMA loading levels is attributed to a nonequilibrium state resulting from a barrier effect. In other words, the relatively high residual concentrations of HDTMA in soil solution after adsorption was complete were controlled by HDTMA adsorption via hydrophobic bonding. The fact that the selectivity coefficient of Na–HDTMA exchange increased as the excess of HDTMA adsorbed by hydrophobic bonding was gradually removed by desorption (Fig. 4) confirmed that the true surface affinity of HDTMA adsorbed on exchange sites at high loading levels is much higher than that indicated by the selectivity coefficients obtained from adsorption.

Kinetic Study of Na–HDTMA Exchange

The data presented in Fig. 6 illustrate two aspects of HDTMA adsorption kinetics. First, Na release consists of both fast and slow steps, which is consistent with other investigations. Using a cationic fluorescent probe [(3-1-pyrenylpropyl)trimethylammonium], Viaene et al. (1987) observed that the added organic cation (up to 0.3 CEC) bound to the external surface of laponite in an immediate adsorption step, followed by slow rearrangement into interlayers. In our soil system, the slow Na release in dilute NaCl solution indicates that Na–HDTMA exchange in the micropores of HIV is slower than that on the external surfaces. In more concentrated NaCl solution, both HIV and the face-to-face aggregated vermiculite will contribute to the slow release of Na. Eliminating the vermiculite interlayer sites made the micropores of HIV the sole source of internal exchange sites and hence the Na release rates no longer depended on ionic strength (Fig. 6). Second, because HDTMA adsorption maximized before maximal Na liberation occurred in all cases, we can conclude that at least a portion of the HDTMA was initially adsorbed by a nonexchange mechanism.

CONCLUSIONS

Stability of organo–clay complexes and organic contaminant sorption efficiency are two major issues in creating an in situ sorbent zone for remediation. Previous studies have demonstrated that the sorption efficiency of HDTMA-modified soil increased with organic cation loading on exchange sites (Lee et al., 1989). Accordingly, it is desirable to maximize HDTMA loadings to achieve the highest sorption efficiency. Due to the complex adsorption mechanisms discussed above, increasing HDTMA loading may either increase or decrease the stability of organo-clay complexes, depending on the exact HDTMA loading level, and soil conditions related to the degree of clay dispersion. When HDTMA ions are first added to soil, they are rapidly adsorbed onto external surface sites through both ion exchange and hydrophobic mechanisms. The adjacent clay sheets then combine to form large aggregates due to the mutual attraction of adsorbed HDTMA tails. At the same time, HDTMA on external surfaces slowly rearrange into interlayers or other internal exchange sites (e.g., exchange sites in micropores of HIV) leaving inorganic cations on the external surfaces of the aggregates. At low HDTMA loadings (<0.6 CEC), conditions leading to clay dispersion (e.g., Na saturation and low ionic strength) prior to HDTMA addition promoted a more uniform distribution of HDTMA in the interlayers, resulting in comparatively lower stability of the organic-clay complexes. Conditions (e.g., high ionic strength or divalent exchangeable cations) leading to clay flocculation before HDTMA was added, limited the accessibility of HDTMA to some exchange sites. This resulted in high stability at low HDTMA loading (<0.6 CEC) due to a dense packing of adsorbed HDTMA in interlayers around edges. However, formation of a HDTMA-derived hydrophobic barrier inhibits hydrated Na or Ca from diffusing out of the interlayers, resulting in inorganic cation entrapment at high HDTMA loading (>0.6 CEC). Under those conditions, hydrophobic bonding became a competitive adsorption mechanism that reduced the overall stability of HDTMA–soil complexes and resulted in higher residual concentrations of HDTMA in solution for a given degree

of saturation of exchange sites. This is because HDTMA binds to soil more strongly via cation exchange than via hydrophobic bonding.

Hexadecyltrimethylammonium adsorption can be reversible or irreversible, depending on the loading levels and the conditions under which HDTMA was adsorbed. At high HDTMA loading levels or in high ionic strength solutions, inorganic–HDTMA exchange demonstrated a degree of irreversibility.

In aquifers, the groundwater composition cannot be readily controlled. Fortunately, major exchangeable cations in most aquifers are divalent cations such as Ca and Mg, which promote the formation of high-stability HDTMA–clay complexes. The most stable HDTMA-modified soil zone is expected whenever oversaturation of soil clays with HDTMA is minimal and the amount of HDTMA adsorbed via hydrophobic bonding is minimal. One way to minimize the excessive HDTMA adsorption in the treated zone may be to inject the HDTMA solution through soil as fast as practically attainable. This will reduce total HDTMA adsorption in the initial step and hence minimize HDTMA remaining hydrophobically bound after rearrangement.

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Cationic Surfactant Sorption to a Vermiculitic Subsoil via Hydrophobic Bonding

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Organoclays formed in soil from the addition of the cationic surfactant hexadecyltrimethylammonium (HDTMA) can effectively immobilize organic contaminants dissolved in water. The adsorption and desorption of HDTMA in a subsoil was studied to determine the stability of surfactant–soil clay complexes as affected by surfactant retention mechanism. We found that HDTMA was initially adsorbed by cation exchange in the interlayer, causing extensive clay aggregation. As the loading increased, HDTMA adsorbed to the external surfaces of aggregates via both cation exchange and hydrophobic bonding, the latter causing positive charge development on surfaces and ultimately clay dispersion. When the equilibrium concentration of HDTMA reached the critical micelle concentration, no further HDTMA adsorption occurred. Desorption of HDTMA was more significant when the HDTMA retention mechanism was hydrophobic bonding. HDTMA adsorption can be affected by cation type as well as the type and concentration of electrolytes, and these factors can be used to minimize the undesirable effects of hydrophobic bonding such as clay dispersion and HDTMA desorption.

Introduction

The sorptive capabilities of subsoils for common organic groundwater contaminants (neutral and anionic) can be enhanced substantially by substituting native inorganic cations of soil clays with organic cations (*1–6*). Sorption coefficients for several organic contaminants such as benzene and alkylbenzenes increased by over 2 orders of magnitude in subsoils exchanged with hexadecyltrimethylammonium (HDTMA) (*2, 5, 6*). Increasing contaminant sorption reduces the transport potential of otherwise mobile species, suggesting the use of this soil modification approach for in-situ remediation of contaminated soils and aquifers (*1, 4, 5*). The feasibility of underground injection of quaternary ammonium cations to create a sorptive zone that would intercept an advancing contaminant plume and immobilize organic contaminants therein has been demonstrated (*7*). Coupling-enhanced contaminant immobilization with subsequent biodegradation within the sorptive zone may provide a comprehensive in-situ remediation technology (*8, 9*).

In the application of in-situ soil modification, quaternary ammonium cationic surfactants would likely be introduced into the subsurface via injection wells. This will likely result in a gradient of surfactant concentrations moving away from the point of injection and uneven HDTMA loadings across the soil profile (*10*). In some regions (e.g., the wetting front) the surfactant loading would be less than the cation-exchange capacity (CEC) of the soil, and the surfactant molecules will be adsorbed at ion-exchange sites (*10*). In other regions, the amount of the surfactant may exceed the CEC, resulting in a portion of the surfactant being adsorbed by hydrophobic interactions (*10–13*).

The surfactant binding mechanism may influence the stability of the organoclay complexes, the expected aqueous phase concentrations of surfactant, and the surface charge characteristics of the organoclays. The HDTMA adsorbed to clay is essentially nontoxic to pollutant-degrading bacteria in soils whereas aqueous-phase HDTMA exerts considerable toxicity (*9*). Extensive hydrophobic bonding of surfactant molecules could result in a buildup of positive charge on the clay surfaces, leading to disaggregation of the organoclays, mobilization of the dispersed particles, and undesirable changes in the hydraulic properties of the treated zone. Therefore, it is important to evaluate the chemistry of surfactant retention via both mechanisms.

Although several studies on adsorption/desorption of amphiphilic organic cations in pure clay systems have been published (*14–19*), only a few studies of this chemistry in soil exist (*10, 20*). To our knowledge, no study of the role that hydrophobic bonding plays in surfactant adsorption in soil has been reported.

Conceptually, hydrophobic bonding includes the mutual attraction between the alkyl chains (tails) of surfactant molecules and the tendency of the hydrophobic tails to be removed from water (*11*). Several models have been proposed to account for nonexchange adsorption of organic cations, all different in the detailed mechanism of hydrophobic bonding. The tail–tail complex model (*17*) assumes nonexchanged organic cations are retained by adhesion of

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their alkyl chains to the tails of the organic cations already bound on exchange sites, as expressed by

$$OcX + Oc^+ = Oc_2X^+ \quad (1)$$

where OcX is the organic cation-surface exchange complex, and Oc^+ is the organic cation. A positive charge is developed for each organic cation adsorbed by hydrophobic bonding, and the charges are neutralized by anions in the diffuse layer.

The neutral site model (*20*) assumes that organic cations are adsorbed as neutral salts on uncharged sites of the mineral surface as expressed by

$$Y + Oc^+ + An^- = YOcAn \quad (2)$$

where Y, An^-, and YOcAn are neutral sites, anions, and the organic cation–surface complexes held via hydrophobic bonding, respectively.

In the hemimicellization model, ionic surfactants form two-dimensional aggregates (hemimicelles) on the adsorbent surface due to the tendency of the hydrophobic moieties of the adsorbing surfactant to remove themselves from the aqueous environment (*21–23*). The experimental data on which this postulate depends are typified by the sodium dodecyl sulfonate (SDS)/alumina system described by Wakamatsu and Fuerstenau (*24*). When the logarithm of the surface concentration of SDS was plotted against the logarithm of its residual concentration, the adsorption curve could be divided into four regions by three critical concentrations. According to these authors, the initial adsorption occurs with no change in ζ-potential, indicating simple ion exchange of SDS with anions in the double layer (region I). Then, at a particular concentration known as the critical hemimicelle concentration, adsorption increases drastically as hemimicelles form on the absorbent; a concomitant change in ζ-potential occurs ultimately reaching zero (region II). A general relationship for surfactant adsorption on a homogeneous surface is (*25*)

$$\log \Gamma_\delta = [\log 2rm - (\Delta G_{ad}/kT)] + m \log C_{eq} \quad (3)$$

where Γ_δ is the total adsorption density of the organic ions; m and r are the average number of ions per hemimicelle and the radius of ions, respectively; C_{eq} is the concentration of organic ion in bulk solution; and ΔG_{ad} is the free energy of hemimicelle adsorption.

At the end of region II, all of the clay surface charge has been neutralized. Further adsorption via hydrophobic bonding occurs as the concentration of SDS increases (region III) (*21*), resulting in negative charge development on the surface which reverses the ζ-potential. As a complete bilayer covers the whole surface, the equilibrium concentration of organic ion reaches the critical micelle concentration (cmc) (region IV), and no further increases in adsorption is observed because only monomer surfactant ions (not micelles) can be adsorbed (*26*).

The two-dimensional condensation model is similar to the hemimicellization model except that the former takes into account normal and lateral potential energies and the entropic changes in the adsorbed layer to predict the formation of "patches" of ionic surfactants. According to this model (*27*), the adsorption isotherm plotted as surface coverage vs log C_{eq} is a step function on homogeneous surfaces. A smooth curve may be possible for soil because the patches formed on highly heterogeneous soil clay surfaces may have so many sizes that the individual step functions are not observable (*27*).

The objectives of this study are (1) to determine the influence of exchangeable cation type, electrolyte type, and concentration on adsorption and desorption of HDTMA in a subsoil; and (2) to test the applicability of four adsorption models for this subsoil using our adsorption/desorption data.

Experimental Section

Soil Samples. Two subsoil samples from Oshtemo (coarse-loamy, mixed, mesic Typic Hapludalfs from Kellogg Biological Station, Hickory Corners, MI) B_t and C horizons were used in this experiment. These soil samples were air-dried and passed through a 1-mm sieve. The subsoils were saturated with either Na^+ or Ca^{2+}. The detailed procedures for soil preparation and characterization and soil properties have been given elsewhere (*10*). Both soils have low organic matter contents (organic carbon ≤0.1%). The CECs are 38 mmol (+) kg^{-1} for Oshtemo B_t and 12 mmol (+) kg^{-1} for Oshtemo C. Major clay types in both samples are vermiculite, hydroxyaluminum-interlayered vermiculite, illite, and kaolinite.

Adsorption of HDTMA. Stock solutions of 0.0225 M hexadecyltrimethylammonium chloride (HDTMA Cl) or hexadecyltrimethylammonium bromide (HDTMA Br) were made by mixing a known amount of unlabeled HDTMA with ^{14}C-labeled HDTMA [as hexadecyltrimethylammonium iodide from American Radiolabelled Chemicals (St. Louis, MO) radiochemical purity >97%, and the specific activity is 2.2×10^{11} Bq $mmol^{-1}$] at a ratio of 10000:1. The stock solution of 0.045 M hexadecyltrimethylammonium sulfate (HDTMA SO_4) was made by mixing equal volumes of 0.09 M HDTMA hydrogen sulfate and 0.09 M hexadecyltrimethylammonium hydroxide with a small amount of the ^{14}C-labeled HDTMA (at a ratio of 5000:1). The pH of this HDTMA SO_4 stock solution was ~6.0.

Soil containing ~0.09 mmol (+) exchangeable cations (e.g., 2.4 g of Oshtemo B) was weighed into 25-mL Corex centrifuge tubes and washed four to six times with dilute Na or Ca salt solutions as follows to remove the extra salts left from soil saturation by Na or Ca (*10*). Dilute salt solutions (25 mL) of NaCl, NaBr, Na_2SO_4, $CaCl_2$, $CaBr_2$, and $CaSO_4$ were pipetted into the tubes. After mixing by vortex for 10 min, the samples were centrifuged, and most of the supernatant (e.g., 22–24 mL) was removed. An equal volume of fresh solution was then added into the tubes and mixed and centrifuged as above. After the last wash, the standardized salt solution and H_2O were pipetted into the tube to achieve salt concentrations ranging from 0.5 to 40 mM, and the sample was mixed well to redisperse the soil. The appropriate volume of stock HDTMA solution, corresponding to 0.1–5.0 times the CEC, was injected into the tube and mixed immediately. The tubes were shaken for 3 days on a reciprocating shaker, then vortexed, and allowed to stand for 2 h. A volume of soil suspension (0.1–5.0 mL) was taken from a fixed depth of 2 cm below the surface for turbidity measurements (described below). The soil suspension was then centrifuged (9680*g* for 25 min) and the ^{14}C activity in supernatant was analyzed by liquid scintillation counting (LSC) (Liquid Scintillation Analyzer, Tri-Carb 1500, Parkard). The amount of HDTMA adsorbed was calculated by the difference between the initial and the final amount of HDTMA in solution.

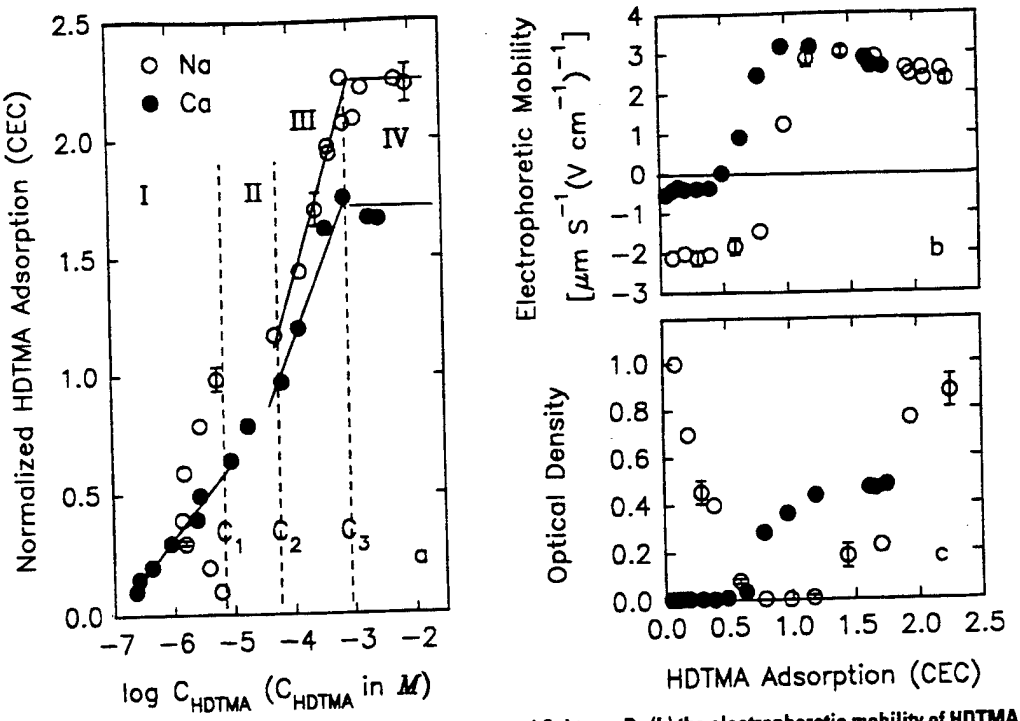

FIGURE 1. (a) Adsorption isotherms of HDTMA Cl in Na- and Ca-saturated Oshtemo B_t; (b) the electrophoretic mobility of HDTMA–soil clays; and (c) the relative turbidity of soil suspension as affected by HDTMA adsorption. The final electrolyte concentrations in all cases were 2.3 mM Cl^-.

Desorption of HDTMA. Supernatant from the adsorption experiments was quantitatively removed from the sample tubes and replaced with deionized water or with $CaCl_2$ or $CaBr_2$ solutions at concentrations of 0.5, 2.5, and 5 mM. The tubes were shaken for 3 days and centrifuged, and the ^{14}C activity in the supernatant was measured by LSC. The HDTMA desorbed was calculated by subtracting the ^{14}C activity derived from the residual solution not removed in the previous step. This procedure was repeated 13–26 times. At the end of the desorption experiment, the amount of adsorbed HDTMA remaining was determined by combustion of the soil in a biological oxidizer (Harvey Biological Oxidizer); the $[^{14}C]CO_2$ evolved was trapped in a mixture of Carbo-sorb II plus aqueous scintillation cocktail, and ^{14}C activity was determined by LSC. Desorption experiments were conducted using soil with two different HDTMA loadings [0.5 CEC where all HDTMA is adsorbed via ion exchange, and 1.98 CEC where HDTMA is adsorbed by ion exchange and hydrophobic bonding (*10*)] to determine the influence of adsorption mechanism on HDTMA desorption.

Microelectrophoresis and Soil Clay Dispersion. The degree of clay dispersion was determined by measuring light adsorption at 375 nm (Spectronic 20, Bausch and Lombe Inc., Rochester, NY) of soil suspension from the HDTMA adsorption experiments as described above. If the suspension was too concentrated, it was diluted with the corresponding supernatant obtained from the adsorption step.

To determine the electrophoretic mobility, the supernatant (~20 mL) of each sample from the HDTMA adsorption experiments was transferred to a Teflon electrophoretic cell. A ~0.01% clay suspension was made by redispersing the soil pellet in the residual supernatant (~2 mL) and transferring ~0.1 mL to the electrophoretic cell. The electrophoretic mobility of 25–50 particles was recorded for each sample using a Zeta Meter (Zeta Meter 3.1, Zeta Meter Inc., New York).

Results and Discussion

Adsorption of HDTMA via Hydrophobic Bonding. *Adsorption Isotherms and Cation Type Effects.* The HDTMA adsorption isotherm can be divided into four distinct regions using three critical concentrations, designated C_1, C_2, and C_3, which are identified by a slope change in the isotherm (Figure 1a) and a change in electrophoretic mobility (Figure 1b). When the equilibrium solution concentration of HDTMA (C_{eq}) was less than C_1 (region I), the shape of HDTMA adsorption isotherms varied, depending on the type of cations initially saturating soil clays. Calcium-saturated soil had a linear isotherm in region I, contrasting to the non-monotonic isotherm for Na-saturated soil. The adsorption mechanism of HDTMA in this region is strictly cation exchange as evidenced by the fact that HDTMA adsorption resulted in equivalent cation release (*10*). The influence of exchange cation type on the shape of adsorption isotherm is related to the degree of clay dispersion. Sodium–clays are well-dispersed in water, and HDTMA can access all exchange sites. As a result, HDTMA cations are distributed randomly on the surfaces and hence more separated from each other. At low loading levels, this distribution minimizes lateral nonpolar interactions between the tails of adsorbed HDTMA molecules, reducing the stability of HDTMA–soil complexes. As HDTMA molecules pack more densely in the interlayers at higher loading levels, increased lateral interactions of adsorbed HDTMAs results in enhanced adsorption and thus the non-monotonic isotherm (Figure 1a). In the Ca–soil suspensions, clay particles are associated through face-to-face aggregation prior to HDTMA addition. This restricts the initial replacement of HDTMA to the outer surfaces and the interlayer regions near the edges of aggregates. This

limited access to some interlayer sites resulted in a dense packing of HDTMA even at low loading levels, manifesting significant lateral HDTMA interactions which were relatively constant in this part of the isotherm.

The adsorption isotherms of HDTMA in both Na- and Ca-saturated soils became linear when C_{eq} was between C_2 and C_3 (region III) and reached plateaus when C_{eq} was greater than C_3 (region IV). In region III, the amount of HDTMA adsorbed exceeded the cation release, which had plateaued, indicating that hydrophobic bonding was the sole mechanism responsible for the increase in HDTMA adsorption. At a given C_{eq}, the amount of HDTMA adsorbed in regions III and IV was greater for Na–soil than Ca–soil (Figure 1a). This is because the amount of cation entrapment is affected by the type of exchangeable cations initially saturating soil clays (*10*). Calcium-saturated clay tends to be flocculated in 0.5 mM $CaCl_2$ solution and forms extensive face-to-face associations (*28*). Access of HTDMA to exchange sites is thereby restricted to near-edge sites. This promotes the entrapment of Ca and results in less HDTMA adsorption.

Region II was a transition between regions I and III. Both adsorption mechanisms are operative in this region. This is indicated by the fact that HDTMA adsorption exceeded cation (Na and Ca) release. Unlike in region III, however, cation release in region II increased with HDTMA adsorption.

Electrophoretic Mobility and Clay Dispersion. The electrophoretic mobility and the degree of dispersion of Na (or Ca)–HDTMA–soil clays support our interpretation of HDTMA adsorption in the four concentration regions. In region I, HDTMA was adsorbed exclusively by cation exchange. The turbidity of the suspension of HDTMA–Na–soil clays decreased with HDTMA loading (Figure 1c), whereas the electrophoretic mobility of the clays remained the same as that of pure Na-saturated soil clays (Figure 1b). The decreased turbidity indicates an increase in clay aggregation as Na was replaced by HDTMA; the constant electrophoretic mobility implies that Na is the only exchange cation on the external surfaces of the aggregates (*29, 30*) and that HDTMA occupies the interlayer regions. For Ca-saturated soil clays, which were flocculated prior to HDTMA addition, increased HDTMA loading in region I has no observable effect on the degree of clay flocculation (Figure 1c). In addition, the electrophoretic mobility of Ca–HDTMA–soil clays, like that of Na-saturated soil clays, was the same as that of pure Ca-saturated soil clays (Figure 1b), indicating Ca as the only exchange cation on the external surfaces of the aggregates. The driving force of this nonuniform distribution of organic and inorganic cations between the interlayer and external surfaces is the hydrophobic interaction between adsorbed HDTMA and the aqueous solutions. Namely, the distribution of HDTMA in the interlayers and Na or Ca on external surfaces minimizes the contact of hydrophobic tails of adsorbed HDTMA with the aqueous phase but maximizes contact between the C-16 alkyl moieties of adsorbed HDTMA molecules. Both effects stabilize the HDTMA–soil complexes.

When HDTMA loading exceeded 0.50 CEC, most of the clay particles flocculated, even in Na-saturated soil, and the turbidity was near zero (Figure 1c). The electrophoretic mobility then increased to zero with a very small increase in HDTMA loading, suggesting HDTMA adsorption on the external surfaces (region II). The steep increase of electrophoretic mobility to the maximum (Figure 1b) as the

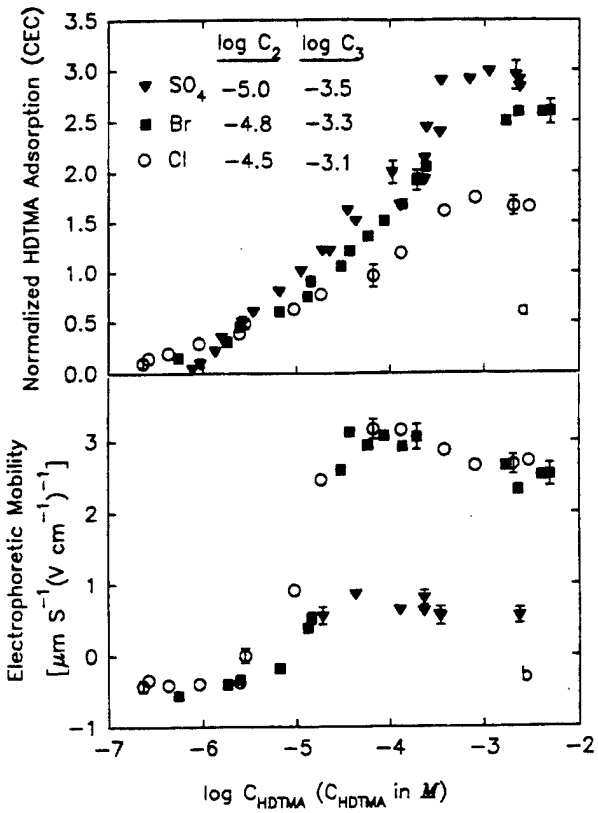

FIGURE 2. (a) Adsorption isotherms of HDTMA in Ca-saturated Oshtemo B_t soil and (b) electrophoretic mobility of HDTMA–soil clay as affected by different companion anions.

total HDTMA loading only slightly exceeded the cummulative cation release suggested that HDTMA adsorption via hydrophobic bonding initially occurred only on the external surfaces of the aggregates.

When HDTMA was added in excess of the CEC (region III), the amount of HDTMA adsorbed on exchange sites remained constant. The increase in total HDTMA retention resulted from additonal adsorption via hydrophobic bonding. The slight change in electrophoretic mobility but drastic increase in the degree of clay dispersion as loading increased for both Na– and Ca–HDTMA–soils in this region (Figure 1b,c) indicates that HDTMA was adsorbed via hydrophobic bonding. This resulted in the development of positive surface charge, which prevented the clays from forming aggregates (e.g., in the case of Na–soil clays) or dismantled the existing aggregates (e.g., in the case of Ca–soil clays).

Anion Type Effects. Anion type lowered C_2 and C_3 in the order: $SO_4^{2-} < Br^- < Cl^-$ (Figure 2). The slope of linear portion of the isotherm in region III and the HDTMA adsorption plateau in region IV also depended on anion type, following in the order of increasing slope and maximum HDTMA adsorption: $SO_4^{2-} > Br^- > Cl^-$.

The limiting factor in ionic surfactant adsorption by hydrophobic bonding is the repulsive force between the charged headgroups (*11*). In the case of HDTMA, anion type effects on the adsorption of HDTMA can be attributed to variation in the ability of different anions to screen the positive charge of the ammonium headgroups. Generally, divalent counterions are more effective in charge screening than monovalent anions, hence HDTMA adsorption via hydrophobic bonding in SO_4^{2-} solutions was higher than

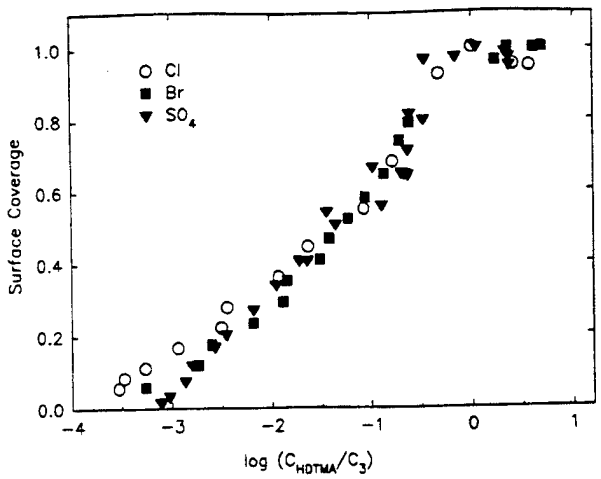

FIGURE 3. Normalized adsorption isotherms for HDTMA in Ca–Oshtemo B_t soil (as shown in Figure 2a).

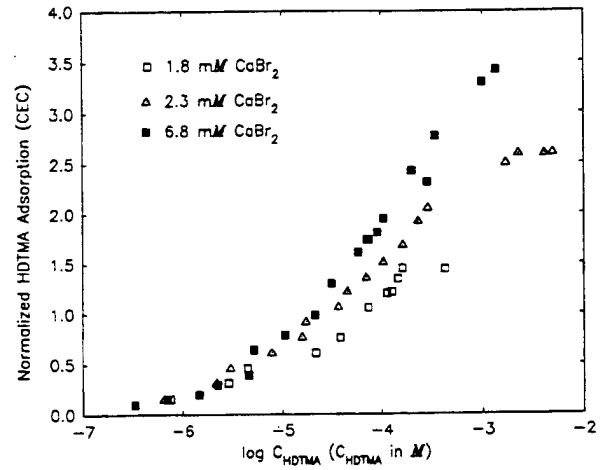

FIGURE 4. Influence of ionic strength on HDTMA adsorption in Ca–Oshtemo B_t.

with monovalent anions. Similarly, Br^- provides more charge screening than Cl^- (*11*), and thus more HDTMA was adsorbed in Br solutions than in Cl solutions.

Differences in charge screening is also a key property responsible for the variation in cmc of ionic surfactants caused by counterion type (*11*). This implies some similarity between micellization of ionic surfactants and their adsorption via hydrophobic bonding. Indeed, in a plot of surface coverage θ (the amount of HDTMA adsorption divided by the plateau adsorption) versus log C_{eq}/C_3 ($C_3 \approx C_{cmc}$), all three curves from Figure 2 converge (Figure 3) at surface coverage >0.4, further supporting the similarity between HDTMA adsorption via hydrophobic bonding and ionic surfactant micellization.

Ionic Strength. Ionic strength had a small effect on HDTMA adsorption in Ca–soil at low loading levels (<0.6–0.7 CEC) where HDTMA adsorption was via cation exchange (Figure 4). This is because HDTMA has much greater affinity for exchange sites than Ca [log $K_{V,Ca-HDTMA}$ is as high as 7–9 (10)]. However, the ionic strength promoted HDTMA adsorption via hydrophobic bonding by lowering the critical concentration C_1, increasing the slope of linear curves in region III, and increasing the maximum HDTMA adsorption in region IV. This is consistent with other investigations, which reported that below the CEC of the clay the adsorption of organic cations (primarily through cation exchange) was not affected much by ionic strength, whereas above the CEC (where both cation exchange and hydrophobic bonding mechanisms are operative) adsorption of organic cations was promoted by high ionic strength (*20, 31*).

Increased HDTMA adsorption at higher ionic strength can be accounted for by a decrease in the thickness of the ionic atmosphere surrounding the positively charged headgroups of HDTMA as the counterion concentration increases and the consequent decrease in electrical repulsion between headgroups in the adsorption layer (*32*). For micelle solutions (e.g., in region IV), this same effect decreased the cmc (*33*), as expressed by

$$\log \text{cmc} = a + b \log C_{An} \qquad (b < 0) \qquad (4)$$

where a and b are constants for a given ionic head at a particular temperature and C_{An} is the total counterion concentration. This explains the decrease of C_3 as ionic strength increased.

For a fixed amount of HDTMA added (2 CEC) to soil (Oshtemo B), the activity of the residual HDTMA (a_{HDTMA}) in region III decreased with increasing in ionic strength (Figure 5):

$$\log a_{HDTMA} = a' + b' \log a_{An} \qquad (b' < 0) \qquad (5)$$

where a' and b' are constants and a_{An} is the activity of anions. This ionic strength effect was more pronounced for Cl^- than Br^- and SO_4^{2-}, indicated by the absolute values of the slopes of the log a_{HDTMA} vs log a_{An} curves: $|b'_{Cl}| > |b'_{Br}| > |b'_{SO_4}|$. As a result of the different slopes, the variation in HDTMA adsorption caused by anion type at a specific concentration was greater in dilute electrolyte solutions where the double layer was fully developed.

Desorption of HDTMA. When the HDTMA–Oshtemo C soil with HDTMA loading of 1.95 CEC was desorbed with 2.5 mM $CaCl_2$, no significant difference between desorption and adsorption isotherms was observed until the HDTMA loading decreased to approximately the CEC (Figure 6), indicating that HDTMA adsorption by hydrophobic bonding is reversible in this subsoil. Desorption hysteresis was observed previously for this soil when all HDTMA was adsorbed via an ion-exchange mechanism (e.g., at HDTMA loading <0.6 CEC) (*10*). The desorption hysteresis in the latter case is attributed to the fact that HDTMA binding to clay via ion exchange is located in the interlayers or interparticle space (in case of illite) of well-aggregated particles. This arrangement excludes full contact of adsorbed HDTMA with the aqueous phase (*10*). The reversibility of HDTMA adsorption via hydrophobic bonding supported our conclusion that HDTMA molecules held by hydrophobic bonding are located on the external surfaces. In addition, changing the desorption solution from 2.5 mM $CaCl_2$ to deionized water increased desorption of HDTMA (Figure 6), showing that low ionic strength promotes desorption of HDTMA adsorbed by hydrophobic bonding.

The influence of adsorption mechanism and anion concentration on the desorbability of HDTMA was further illustrated in Oshtemo B soil (Table 1). At a HDTMA loading of 0.49 CEC, only 6.3% or less of the adsorbed HDTMA was desorbed in 13 consecutive washes (corresponding to ~600 pore volumes) and ionic strength had a negligible influence on the desorption. At a HDTMA loading of 1.95 CEC, the fraction of HDTMA desorbed in 13 washes varied from 8

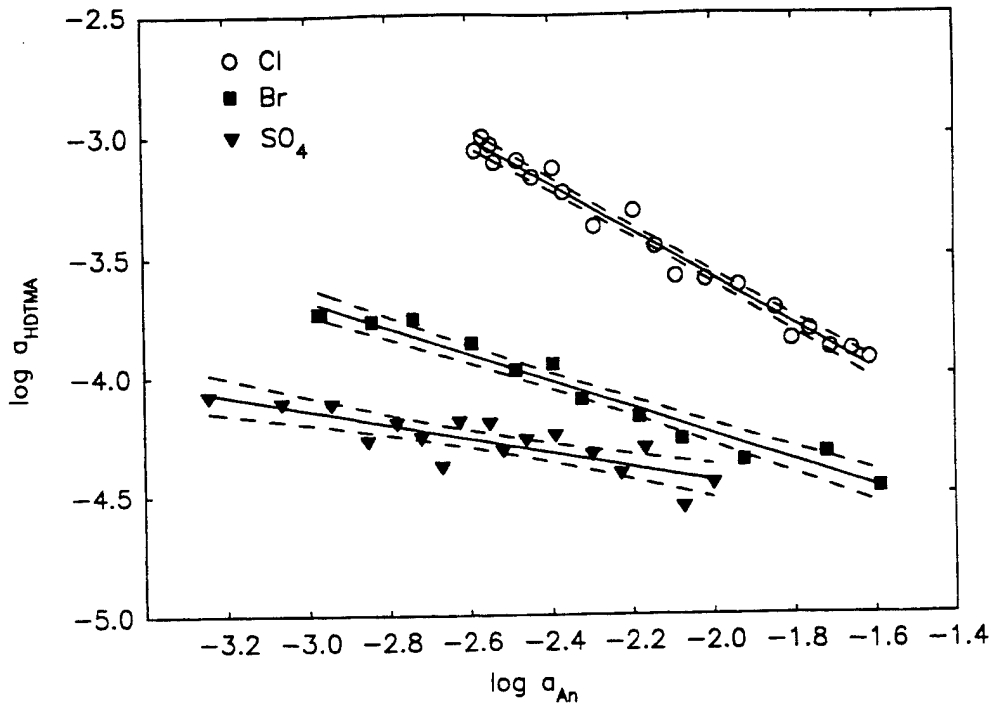

FIGURE 5. Influence of ionic strength on activity of residual HDTMA in NaCl, NaBr, and Na_2SO_4 solutions after a fixed amount of HDTMA (2 equiv of the soil CEC) was added to Na−Oshtemo B_t soil suspension. Dashed lines indicate the 95% confidence intervals.

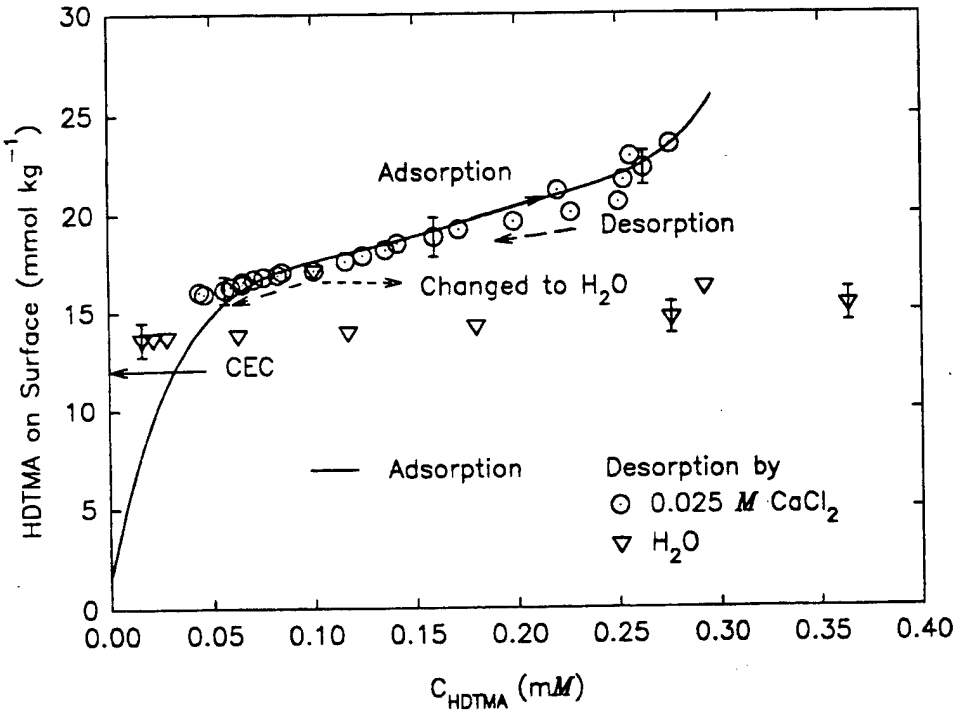

FIGURE 6. Comparison of adsorption with desorption of HDTMA Cl in Ca−Oshtemo C soil in 0.025 M $CaCl_2$. Notice that the desorption increased when the leaching solution change from 0.025 M $CaCl_2$ to H_2O.

to 29% and was strongly dependent on ionic strength. The HDTMA desorption was highest in H_2O and was reduced significantly in low ionic solutions. This is consistent with the results from our adsorption experiments that the repulsive forces between the positively charged headgroups increased as the thickness of the double layers around the headgroups increased in low ionic strength solutions.

The amount of HDTMA remaining sorbed (Figure 6 and Table 1) was calculated by subtracting the cumulative removal of HDTMA by consecutive washings from the total amount of HDTMA adsorbed. Those results were checked by the combustion of soil samples after the last wash and were in good agreement (average recovery rate is 97% with a range between 87 and 103%).

Comparison with Adsorption Models. There are some inconsistencies between the tail-tail complexation model predictions manifested by eq 1 and our experimental data. First, by assuming one (HDTMA adsorbed via ion exchange)

TABLE 1

Fraction of HDTMA Remaining after HDTMA–Oshtemo B_t Soil Was Exposed to Water and $CaBr_2$ Solutions[a]

	initial HDTMA loading = 0.49 CEC				initial HDTMA loading = 1.95 CE			
	$CaBr_2$ concn (mM)				$CaBr_2$ concn (mM)			
no. of washes	5	2.5	0.5	water	5	2.5	0.5	water
1	0.998	0.998	0.998	0.999	0.984	0.984	0.977	0.971
2	0.997	0.998	0.997	0.996	0.978	0.975	0.953	0.927
3	0.995	0.996	0.996	0.993	0.972	0.967	0.935	0.889
4	0.990	0.994	0.994	0.988	0.967	0.960	0.901	0.857
5	0.987	0.992	0.991	0.986	0.963	0.954	0.889	0.831
6	0.982	0.988	0.988	0.981	0.957	0.947	0.876	0.806
7	0.997	0.982	0.986	0.973	0.951	0.940	0.863	0.784
8	0.973	0.979	0.983	0.969	0.947	0.934	0.851	0.766
9	0.967	0.974	0.980	0.964	0.942	0.927	0.840	0.751
10	0.963	0.970	0.978	0.957	0.937	0.920	0.828	0.737
11	0.958	0.963	0.975	0.952	0.932	0.914	0.818	0.728
12	0.951	0.955	0.970	0.944	0.927	0.907	0.806	0.718
13	0.942	0.943	0.965	0.936	0.921	0.900	0.794	0.708
as detd by combustion[b]	0.818	0.868	0.947	0.930	0.919	0.923	0.805	0.669

[a] Solid to liquid ratio = 1:10 (w/w). [b] Fraction of HDTMA remaining in soil after the 13th wash.

to one (HDTMA adsorbed via hydrophobic bonding) complexation, the model predicts the maximum organic cation adsorption as twice the CEC. But maximum adsorption of HDTMA in this soil varied from 1.5 CEC to more than 3.4 CEC, depending on the initial degree of clay dispersion, companion anion type, and ionic strength. Also the model assumes that all counterions reside in the diffuse layer by using the Gouy–Chapman model to relate surface potential to the organic cation adsorption (*17*). Therefore, it predicts no effects from anions of similar charge (e.g., Cl^- vs Br^-) on adsorption of HDTMA, contradicting our experimental observations.

Although the neutral site model may account for anion type effects, it does not account for the surface charge reversal and dispersion of HDTMA–clay at high HDTMA loading. This is because there is no mechanism in this model for positive charge development on the surface. In addition, multiple types of neutral sites with arbitrarily chosen bonding constants have to be assumed to fit the experimental data to the model. For example, up to five different types of neutral sites (nine fitting parameters) have been assumed by Brownawell et al. (*20*) for a sandy soil. The use of too many flexible parameters makes it difficult, if not impossible, to test the validity and applicability of this model in our vermiculitic soil.

As we mentioned earlier, the hemimicellization model has been applied successfully to account for ionic surfactant adsorption even below the ion-exchange capacity of metal oxides (*24*). Formation of hemimicelles at low organic cation loading levels (e.g., $C_{eq} < C_1$) is not evident in our soil samples. For example, in dilute NaCl solutions, the slope of HDTMA adsorption isotherms at HDTMA loading ≤1 CEC (region I) varied with HDTMA loading level and ionic strength (Figure 1), suggesting that a simple log–log linear relationship between the amount of surfactant adsorbed and its equilibrium concentration in water (see eq 3), such as that observed for hemimicellization on oxides (*24*), does not apply in organic–inorganic exchange involving swelling clays. Other investigators (*20*) have observed the log–log relationship for dodecyl pyridium in montmorillonite. However, the isotherm slope, which is equivalent to the number of surfactant molecules per hemimicelle, is too low (<2) to support the existence of hemimicelles, and there is no slope rise analogous to region II of SDS adsorption on oxides (*24*).

Both the hemimicellization and two-dimensional condensation models are qualitatively consistent with the main characteristics of HDTMA adsorption at high loading levels (region III of Figure 1) in our soil, namely, the shapes of the adsorption isotherms, positive charge development and clay redispersion, maximum adsorption much beyond 2 CEC, and anion type and ionic strength effects. These models share the common hypothesis that HDTMAs adsorbed via hydrophobic bonding (either through hemimicelle formation or two-dimensional condensation) are arranged on external surfaces with the positively charged headgroups oriented toward bulk water. Because the driving force and limiting factor of hemimicellization or two-dimensional condensation are very similar to those for micellization of surfactants, the effects of anion type and ionic strength on HDTMA adsorption beyond CEC were similar to those on micellization. In addition, this kind of retention is not site-limited. Therefore, the maximum adsorption does not depend on the density of specific sites, but rather will be determined by the available external surface area and packing density of HDTMAs in the adsorption layer which, in turn, depends on how well the charge of headgroups is shielded by anions. Because positive charge on the clay particle was detected by electrophoresis and because Br^- and Cl^- differentially affected HDTMA adsorption, it is reasonable to conclude that positive charges on the headgroups of HDTMAs held via the non-ion-exchange mechanism were neutralized by anions both directly binding to the headgroups and those swarming in the diffuse layer.

A major strength of hemimicellization model is that it can account for the change of adsorption isotherm in regions II–IV. When HDTMA sorption via hydrophobic bonding commenced at C_1, the initial amount sorbed was low. As C_{eq} reached C_2, HDTMA adsorption increased dramatically, and the adsorption isotherms became linear, leaving an apparent inflection point in the adsorption curve (Figure 1). By the hemimicellization model, this point corresponds to the critical hemimicelle concentration (CHMC). Both C_3, which corresponds to the the cmc of

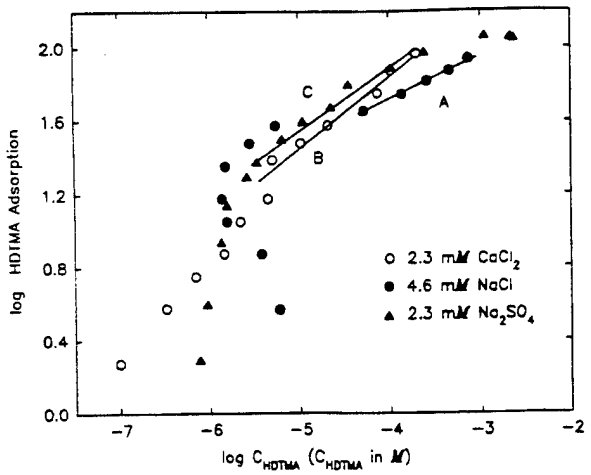

FIGURE 7. Illustration of simple linear relationship between logarithm of HDTMA adsorption and logarithm of HDTMA solution concentration at high HDTMA loadings (lines A, B, and C) in Oshtemo B_t soil.

HDTMA at a specific ionic strength, and C_2 decreased with ionic strength and changed with different companion anions.

Although the hemimicellization model adequately describes some characteristics of HDTMA adsorption beyond the CEC, there are some quantitative inconsistencies. Although HDTMA adsorption via hydrophobic bonding obeys the simple linear log–log relationship as indicated by eq 3, the slope of the curve was less than 2 (Figure 7), thus contradicting the concept of a hemimicelle. The cause of this inconsistency may arise from the contribution of the repulsive force of headgroups to the total adsorption free energy (ΔG_{ad}), which could lead to a decrease in slope if this contribution changes as organic cation loading changes.

As we mentioned earlier, a typical curve of two-dimensional condensation model will be a step function corresponding to the formation of a surfactant "patch" on the surface. The smooth curve observed in Figure 3 can be still explained using this model by invoking surface heterogeneity, which controls the sizes of the patches (*27*). That is, the patches formed on highly heterogeneous soil clay surfaces have so many sizes that the individual step functions were not distinguishable (*34*).

Summary

Adsorption isotherms of HDTMA in this subsoil can be divided into four different regions by three critical concentrations of HDTMA. At low loading levels ($C_{eq} < C_1$), HDTMA was adsorbed by cation exchange only. The HDTMA is primarily adsorbed in the interlayers, and inorganic cations tend to occupy the external surfaces. As the first critical concentration of HDTMA (C_1) was reached, HDTMA adsorption via hydrophobic bonding commenced. Those HDTMAs were distributed only on the external surfaces with their charges balanced by anions bound directly to or swarming around the headgroups. The electrophoretic mobility of HDTMA–clays maximized at the end of this region while the clay remained flocculated. As HDTMA concentration increased to C_2 (the critical hemimicellization concentration), the adsorption isotherm of HDTMA became linear and rose sharply. The electrophoretic mobility decreased slightly and the clay started to disperse. This continued until the concentration of HDTMA reached the cmc (C_3). Thereafter, no change in HDTMA adsorption or electrophoretic mobility was observed.

The degree of clay dispersion (as determined by inorganic exchange cations) before the HDTMA was added influenced HDTMA adsorption by affecting the degree of cation entrapment and the surface area available to HDTMA. Different anions having different charge screening strength and anion type thus affected HDTMA adsorption. The order of increasing charge screening strength and increasing HDTMA adsorption (via decreasing C_2, increasing the slope of adsorption isotherms in region III, and the HDTMA adsorption plateau in region IV) was $SO_4^{2-} > Br^- > Cl^-$. This anion type effect was more profound in dilute electrolytes where the double layer was fully developed. Ionic strength affects the thickness of the double layer surrounding the headgroups of HDTMA adsorbed by hydrophobic bonding; increasing the ionic strength thus increased HDTMA adsorption. The desorption of HDTMA adsorbed via hydrophobic bonding showed no hysteresis. Increasing the ionic strength of leaching solutions decreased the desorption of HDTMA.

All of the above data supported the common hypothesis of both hemimicellization and two-dimensional condensation models that HDTMA molecules adsorbed beyond the CEC were aggregated on the external surfaces with the headgroups oriented toward the bulk aqueous phase and their charge balanced by counterions in both the Stern layer and the diffuse layer. However, both models need further modification before they can be applied to soils.

Two undesirable manifestations of HDTMA adsorption via hydrophobic bonding are the tendency for desorption in water and clay dispersion. In in-situ soil modification, HDTMA adsorption via hydrophobic bonding is likely to occur near the point of injection. However, our data suggest that HDTMA adsorption via hydrophobic bonding can be minimized by controlling companion anions (e.g., using HDTMA Cl instead HDTMA Br) and by lowering the ionic strength of the electrolytes during modification. Related issues that need systematic study are the influence of oversaturation of soil by HDTMA on contaminant sorption effeciency and hydraulic properties of HDTMA-modified soils.

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APPENDIX B

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Heterotrophic Activity of Microorganisms in Soils Treated with Quaternary Ammonium Compounds

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In situ soil modification using quaternary ammonium compounds (QACs) enhances the immobilization of organic contaminants. The feasibility of coupling contaminant immobilization with subseqeunt bioremediation was assessed in biodegradability tests of ^{14}C-labeled organic compounds in QAC-treated soils. Aqueous hexadecyltrimethylammonium (HDTMA) bromide added to soils caused increased lag periods and decreased rates and extents of mineralization of test compounds as a result of selective toxicity toward Gram-negative soil microorganisms. Toxic effects were more pronounced at higher HDTMA treatment levels and with more complex test substrates. Once bound to soil or clay, HDTMA toxicity was greatly reduced. Hence, biodegradation improved when HDTMA was introduced to soil in a prebound form, when sterile soils preequilibrated with HDTMA were reinoculated with active soil organisms, or when an HDTMA-degrading bacterial isolate was introduced to scavenge residual, unbound HDTMA. Dioctadecyldimethylammonium (DODMA) bromide was approximately 10-fold less toxic to the Gram-negative organism, *Pseudomonas putida*, and was less inhibitory to native naphthalene-degrading organisms in soil than HDTMA.

Introduction

Recently, we have shown that the sorptive capabilities of soils and subsoils for organic contaminants can be greatly enhanced by substituting native inorganic exchange cations of soil clays with quaternary ammonium compounds (QACs) of the form $[(CH_3)_3NR]^+$ or $[(CH_3)_2NRR']^+$, where R and R′ are large ($>C_{10}$) alkyl hydrocarbon chains (*1-4*). Sorption coefficients for several common organic groundwater contaminants increased by over 2 orders of magnitude in B-horizon soils treated with hexadecyltrimethylammonium (HDTMA) bromide. The HDTMA-derived sorptive phases functioned as highly effective partition media for such contaminants. With HDTMA-modified subsoils, carbon-normalized sorption coefficients (K_{oc}'s) for nonionic organic contaminants are approximately 10-fold greater than coefficients for sorption to natural soil organic matter and approximate K_{ow} values for the solutes. This indicates that the solvency of sorbed surfactant phases for nonionic organic compounds is significantly greater than that of the more polar and rigid matrices comprising natural soil organic matter.

These results suggest the possibility of managing contaminant plumes by creating sorptive zones *in situ* via underground injections of QACs (*1, 2, 5*). HDTMA generally has much greater affinity for cation-exchange sites than inorganic cations native to soil minerals and organic matter; hence, a fully HDTMA-saturated soil can be created with very low residual HDTMA concentrations in solution (*6, 7*). The feasibility of creating such a sorbent zone that would intercept an advancing contaminant plume and immobilize contaminants contained therein has been recently demonstrated (*8*). Although this approach would effectively reduce downgradient contaminant concentrations in groundwater, coupling contaminant immobilization with subsequent bioremediation would provide a more comprehensive approach for *in situ* restoration (*5*). To examine the feasibility of this approach, the toxicity of QACs to soil microorganisms was studied.

QACs are active biocidal agents used widely as disinfectants (*9, 10*). Diquat and paraquat are herbicides in which the ammonium cation is part of a pyridinium ring structure. The exchange of these cations on mineral surfaces reduces their availability for plant uptake (*11*) and biodegradation (*12*) and attenuates their toxicity toward plant and animal targets (*11, 13*). As surfactants used in detergents, fabric softeners, and hair conditioners, QACs are common components of domestic waste waters. U.S. consumption of alkyldimethylbenzylammonium compounds, representing just one type of QAC, was estimated to be 20–25 million lb in 1979 (*14*). In receiving waters, the toxicity of these compounds toward bacteria and algae is inversely related to the concentration of suspended particulate material (*15*), and microbial adaptation and cation degradation are commonly observed in environments with a history of exposure (*16–18*).

Previous work has shown that QACs are potent biocides which bind to proteins and nucleic acids, disrupt membrane integrity, and cause leakage of cytoplasmic ions and macromolecules (*19*). However, the concentrations at which different organisms are susceptible varies (*20*), and plasmid-conferred resistance to these compounds has been reported for certain bacteria (*21*). Furthermore, organisms able to utilize HDTMA as a sole carbon and energy source have recently been isolated (*22, 23*). In this paper, we show that, in the unbound form, HDTMA is toxic and adversely impacts the heterotrophic activities of aerobic soil microorganisms. Once adsorbed to mineral phases, however, toxic effects are largely eliminated.

Materials and Methods

Soils. Two soils were used in these studies (Table 1). The Marlette soil is a fine-loamy, mixed, mesic glossoboric hapludalfs collected from the Crop and Soil Sciences farm at Michigan State University. Both the A and B_t horizons were used in these studies. Aquifer sediment (approximate depth, 7.5 ft) collected from a jet fuel-contaminated site in Alaksa and referred to as the Eielson aquifer material was also studied. Soils were stored at 5 °C upon collection and were air-dried and sieved (2 mm) before use. Soils were characterized with respect to cation-exchange capacity (CEC) by Ba displacement of Mg-saturated soils (*24*), organic carbon content by microcombustion (Huffman Laboratories, Golden, CO), and particle size distri-

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Table 1. Characteristics of Soils Used in This Study

soil	CEC[a]	% sand	% silt	% clay	% OC[b]
Marlette A	11.5	54.9	35.7	9.4	2.2
Marlette B_t	12.8	51.5	31.3	17.2	0.6
Eielson	8.0	32.9	62.8	4.3	0.85

[a] Cation-exchange capacity (mequiv/100 g). [b] Percent organic carbon (soil dry weight).

bution by the hydrometer method (25). Isotherms for the sorption of HDTMA to soils suspended in phosphate-buffered saline were measured by the batch method (26) using ^{14}C-labeled HDTMA and liquid scintillation counting to determine equilibrium aqueous concentrations. Sorbed HDTMA concentrations were determined by difference.

Radiochemicals. Radiolabeled substrates used in toxicity assays included [U-^{14}C]D-glucose (8.7 mCi mmol^{-1}), [ring-U-^{14}C]toluene (51.5 mCi mmol^{-1}), [ring-U-^{14}C]2,4-dichlorophenoxyacetic acid (12.8 mCi mmol^{-1}), [1-^{14}C]-naphthalene (10.1 mCi mmol^{-1}), [9-^{14}C]phenanthrene (13.1 mCi mmol^{-1}), and [ring-U-^{14}C]salicylate (7.6 mCi mmol^{-1}). All compounds were obtained from the manufacturer (Sigma) at >98% purity and were used without further purification. [^{14}C]HDTMA, labeled in the carbon distal from the ammonium headgroup, was obtained from Moravek Biochemicals, Inc. (Brea, CA), and had a radiochemical purity >97% and a specific activity of 55 mCi mmol^{-1}.

Quaternary Ammonium Compounds. The QACs used in this study were HDTMA bromide (Sigma; critical micelle concentration, 9.4×10^{-4} M), nonyltrimethylammonium bromide (NTMA, Kodak), dodecyltrimethylammonium bromide (DdTMA, Kodak), cetylpyridinium bromide (CPB, Sigma), and DODMA bromide (Kodak; critical micelle concentration, 4.3×10^{-5} M).

Mineralization Assays. The influence of HDTMA on the heterotrophic activities of soil microbial populations was determined by incubating soil slurries with ^{14}C-labeled test substrates with or without the addition of HDTMA in modified Bartha–Pramer flasks (27). The flasks consisted of 250-mL Erlenmeyer flasks with Teflon-lined screw caps and Teflon-capped crimp-sealed side-arm reservoirs. The conical side-arm reservoirs were equipped with 18-gauge luer lock needles inserted through the septa and fitted with a short length of Teflon tubing at the tip to allow quantitative recovery of $^{14}CO_2$ trapping fluid (2 N KOH) from the reservoirs.

Solutions of ^{14}C-labeled substrates and/or HDTMA were prepared in phosphate-buffered saline (PBS; 8.5 g of NaCl, 0.3 g of KH_2PO_4, and 0.6 g of Na_2HPO_4/L of distilled water, pH 7.0) at the specified concentrations. Sufficient ^{14}C-labeled substrate was added to the unlabeled compound to attain total activities of approximately 2.5×10^5 dpm per flask. Added activities were determined by counting 1-mL aliquots of the solutions prior to addition to the flasks. Substrate solutions (50 mL) were amended with HDTMA in quantities calculated to satisfy fixed percentages (0, 30, 50, or 70) of the soil CEC before addition to duplicate flasks containing 10 g of air-dried soil.

Soil slurries were incubated at room temperature on a rotary shaker (200 rpm). At intervals, the $^{14}CO_2$ trapping solution (1 mL) was quantitatively removed by a syringe and transferred to a scintillation vial containing 7.5 mL of scintillation cocktail. Activities of samples were determined by liquid scintillation counting (Packard 1500 Tri-Carb liquid scintillation analyzer). Counts were converted to disintegrations per minute by external standard quench correction, and the cumulative $^{14}CO_2$ production was plotted as a function of time. For long-term experiments, air was periodically introduced into the flasks by loosening the screw cap and drawing air past the KOH solution by use of a 25-mL syringe connected to the side-arm canula.

The influence of adding HDTMA in a prebound form to fresh soil on the mineralization of 2,4-D was also measured. Marlette B_t horizon soil was sterilized by autoclaving (1 h each on three consecutive days), and equilibrated (overnight shaking) with HDTMA at 70% of the CEC in PBS. The treated soil was centrifuged (7000g, 30 min) to remove unbound HDTMA, and the pellet was air-dried. Adsorbed HDTMA concentrations were determined in replicate samples prepared in an identical manner but spiked with [^{14}C]HDTMA at 5000 dpm mL^{-1}. One-milliter aliquots of the supernatants of labeled samples were analyzed by liquid scintillation counting to determine the bound HDTMA concentration by difference. HDTMA-modified smectite clay (Clay Minerals Society reference material, SWy-1, <2 μm fraction obtained by settling) was prepared in an analogous manner. The mineralization of ^{14}C-labeled 2,4-D (10 μg mL^{-1}) in Marlette A horizon soil slurries (10 g per 50 mL) was compared in systems receiving sterile HDTMA-treated Marlette B_t soil, sterile HDTMA-treated smectite, or an equivalent mass of free HDTMA calculated to satisfy 70% of the soil CEC.

Similarly, the effect of prebound vs free HDTMA on the mineralization of naphthalene by Eielson aquifer microorganisms was studied. A total of 50 mL of a PBS solution containing HDTMA (50% of the CEC) and naphthalene (2.5×10^5 dpm ^{14}C-labeled naphthalene, 2 μg mL^{-1} total concentration) was added to three sets of duplicate biometer flasks, each containing 10 g of sterilized Eielson aquifer sediment. A fourth set of flasks received naphthalene but no HDTMA. After equilibration (24 h with shaking), unbound HDTMA was scavenged from one set of flasks by the addition of sterile smectite clay (89 mg) and from another set by the addition of a pure culture of an HDTMA-degrading bacterium (final density, 10^8 cells mL^{-1}). After 3 days, 1 g of fresh Eielson aquifer material was added to all flasks as a source of inoculum, and the mineralization of naphthalene was followed. Control flasks amended with unlabeled naphthalene and [^{14}C]HDTMA were included to estimate the mineralization of HDTMA in flasks receiving HDTMA-degrading bacteria. This organism did not degrade naphthalene.

The toxicity of DODMA was tested in naphthalene (1 μg mL^{-1}) and salicylate (5 μg mL^{-1}) mineralization assays. Eielson aquifer sediment was treated with unbound DODMA at 50% of the soil CEC and production of $^{14}CO_2$ was monitored over time.

Enumeration of Soil Bacteria. The effect of HDTMA on the viability and diversity of the aerobic soil bacterial community was examined. Samples of 10 g of freshly collected and briefly air-dried Marlette A horizon soil and Eielson aquifer material were treated with HDTMA (in 50 mL of PBS) at 50% of the soil CEC by shaking (200 rpm) for 1 h at room temperature. Slurries were blended briefly and serially diluted in PBS. Dilutions were spread-plated in triplicate onto nutrient, PTYG, 1:20 PTYG,

and tap water agars. The nutrient agar consisted of 4 g of nutrient broth (Difco) and 15 g of agar (Difco)/L of DW. PTYG agar was comprised of 10 g each of D-glucose (Baker) and yeast extract (Difco), 5 g each of peptone (Sigma) and trypticase soy broth (BBL), 0.6 g of $MgSO_4 \cdot 7H_2O$, 0.07 g of $CaCl_2 \cdot 2H_2O$, and 15 g of agar/L of DW. The 1:20 PTYG agar medium was a 20-fold dilution of PTYG medium maintaining the agar at 15 g/L. Tap water agar consisted of 15 g of agar/L of tap water. Each medium contained cycloheximide (100 mg L^{-1}, Sigma) to inhibit fungal overgrowth on plates.

Plates were incubated in the dark at 25 °C for 2–3 weeks and compared to control plates (from soils treated identically except for the ommission of HDTMA) with respect to colony numbers, size, shape, and color. Selected colonies from treated samples were streaked onto homologous media for isolation and were subsequently cultured on PTYG agar.

Characterization of Isolates. Eleven isolates from HDTMA-treated Marlette A horizon soil were chosen for further study. Additional isolates obtained from the American Type Culture Collection were also examined including *Micrococcus luteus* (ATCC 4698), *Pseudomonas putida* (ATCC 17484), *Rhodococcus rhodochrous* (ATCC 14347), and *Arthrobacter globiformis* (ATCC 8010). An *Alcaligenes* sp. (strain NP-Alk; *28*) was also included.

To identify unknown isolates, cells were examined by phase contrast micropscopy (motility, endospore production, cell morphology), biochemical tests (catalase and oxidase) and Gram reaction. Spore formation was confirmed by the survival of cell suspensions heated to 80 °C for 10 min with subsequent plating onto PTYG agar plates. The carbon source utilization patterns of isolates were determined by automated Biolog analysis (Biolog Inc., Hayward, CA) of cells following growth on trypticase soy agar. Carbon source utilization patterns were cross-referenced to a computer library for known species, and similarity coefficients to the best matches were calculated. Similarity indices of unknown isolates to known species were also determined on the basis of gas chromatographic analysis of fatty acid methyl ester profiles of organisms following growth on trypticase soy agar using the automated Microbial Identification System (MIDI, Newark, DE).

The toxicity of HDTMA to the above organisms was determined by incubating washed suspensions of late log phase cells (10^8 mL^{-1}) in PBS solutions containing a range of HDTMA concentrations for exactly 1 h. Following treatment, cells were diluted serially in PBS, plated onto PTYG or nutrient agar (without cycloheximide), and incubated at 20 °C for 1 week. Colony counts of treated samples were compared to untreated controls to obtain estimates of the percent survival. Linear regression (r^2 values ≥ 0.975) of the percent survival as a function of the log [HDTMA concentration] was used to obtain estimates of the LC_{50} for each isolate, i.e., the HDTMA concentration at which 50% survival occurred. The time course of HDTMA toxicity and the relative toxicities of other QACs (NTMA, DdTMA, CPB, and DODMA) were also tested against *P. putida*.

Results

The characteristics of the soils used in these studies are shown in Table 1. The Marlette A and B_t horizon soils had similar CECs with the clay mineral fraction contributing relatively more of this capacity in the B_t horizon and the organic fraction contributing more in the A horizon. The Eielson aquifer material was a fairly fine-grained soil due to its high silt content. It also contained appreciable organic carbon considering its subsurface origin. Sorption of HDTMA to all soils yielded characteristic (*3, 4*) H (high-affinity) type isotherms (not shown) with asymptotes (equilibrium binding capacities) approximating the CECs of the soils. Such isotherms indicate that HDTMA exchanges readily with inorganic cations on organic matter and mineral surfaces.

The apparent toxicity of unbound HDTMA toward the heterotrophic microbial community in Marlette A horizon soil was dependent on the test substrate and HDTMA treatment level. With glucose, only minoor lags in the onset of mineralization were observed at the 30 and 70% treatment levels with no reductions in the extent of mineralization compared to the untreated control (Figure 1a). The lags prior to the mineralization of salicylate (70% level) and 2,4-D (30% level) were about double those in the controls (Figure 1b,c). The extent of salicylate mineralization also decreased as the added amount of HDTMA increased. No reduction in the extent of 2,4-D mineralization occurred at the 30% treatment level, but less than 2% was mineralized after 800-h incubation at the 70% treatment level (Figure 1c).

Similar results were observed in the mineralization of aromatic substrates by Eielson aquifer microorganisms. Figure 2 shows that although the untreated sediments were well adapted for degradation of these compounds, the toxic effects of HDTMA became more apparent as the substrate complexity increased from toluene to naphthalene to phenanthrene. For toluene and naphthalene, the degradative capability of the soil microbial population was preserved at all treatment levels, but the rates and extents of mineralization were reduced (Figure 2a,b). Phenanthrene mineralization in Eielson aquifer material was completely inhibited at all HDTMA treatment levels (Figure 2c). Spread plates of diluted slurries from these flasks following incubation (~8 weeks) showed that phenanthrene-degrading organisms (clearing zones in a crystalline phenanthrene overlay; *29*) were present in high numbers ($>10^4$ mL^{-1}) in control slurries but were absent in HDTMA-treated slurries.

The toxic effects of HDTMA were confirmed in plating experiments in which the Marlette A horizon soil and the Eielson aquifier material were exposed to HDTMA at 50% of their respective CECs for 1 h. This treatment resulted in 9- and 15-fold reductions (average of four media types) in the numbers of culturable bacteria in the Marlette and Eielson soils, respectively. Diversity of the culturable population was also reduced by at least 50% in both soils as estimated by the numbers of colony morphotypes.

Eleven distinct morphotypes from the treated Marlette soil were isolated for further characterization. Nine of the eleven isolates gave Gram-positive reactions, and seven of these produced visible endospores (Table 2). These seven organisms matched most closely to various *Bacillus* spp. Oxidase and catalase reactions (not shown) were consistent with the fatty acid methyl ester designations of all isolates. From these results, it appears that HDTMA is more toxic to Gram-negative than to Gram-positive organisms. The predominance of *Bacillus* spp. among HDTMA survivors suggests that these organisms may have existed as spores at the time of HDTMA treatment.

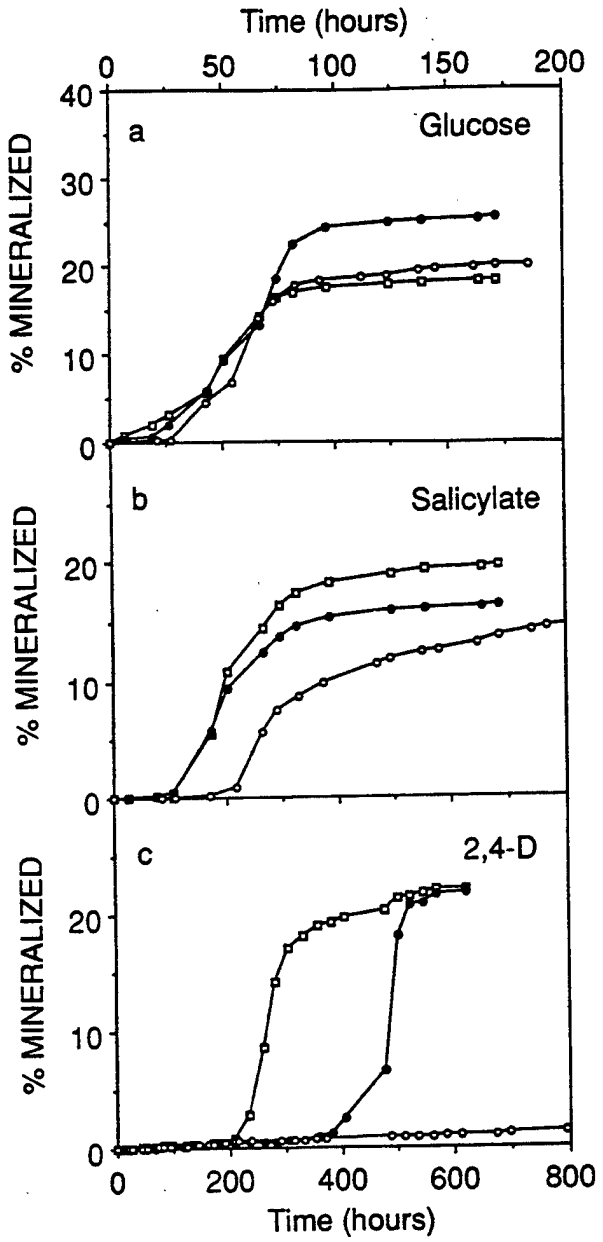

Figure 1. Time courses for the mineralization of glucose (a, 200 μg mL^{-1}), salicylate (b, 5 μg mL^{-1}), and 2,4-D (c, 10 μg mL^{-1}) in Marlette A soil slurries (10 g soil, 50 mL of PBS) treated with HDTMA at 0 (controls, open squares), 30% (solid circles), or 70% (open circles) of the cation-exchange capacity. The upper time scale applies to panels a and b; the lower time scale applies to panel c.

The Gram-positive *Bacillus* isolates gave LC_{50} values for HDTMA ranging from 28 to 514 μM (Table 2). Phase contrast microscopic examination of the cultures prior to toxicity tests showed that vegetative cells rather than spores predominated in the late log-phase cultures used in these assays. In contrast, three of the Gram-negative organisms tested had LC_{50} values below 10 μM. Isolate P, a prodigious slime-producing organism identified as an *Enterobacter* sp. by fatty acid methyl ester analysis, was relatively resistant to HDTMA (LC_{50} = 51 μM). The only Gram-positive organism with an LC_{50} below 10 μM was the type strain of *A. globiformis*. Attempts to relate LC_{50} values to other measurable cell parameters such as cell surface hydrophobicity and HDTMA binding capacity proved unfruitful.

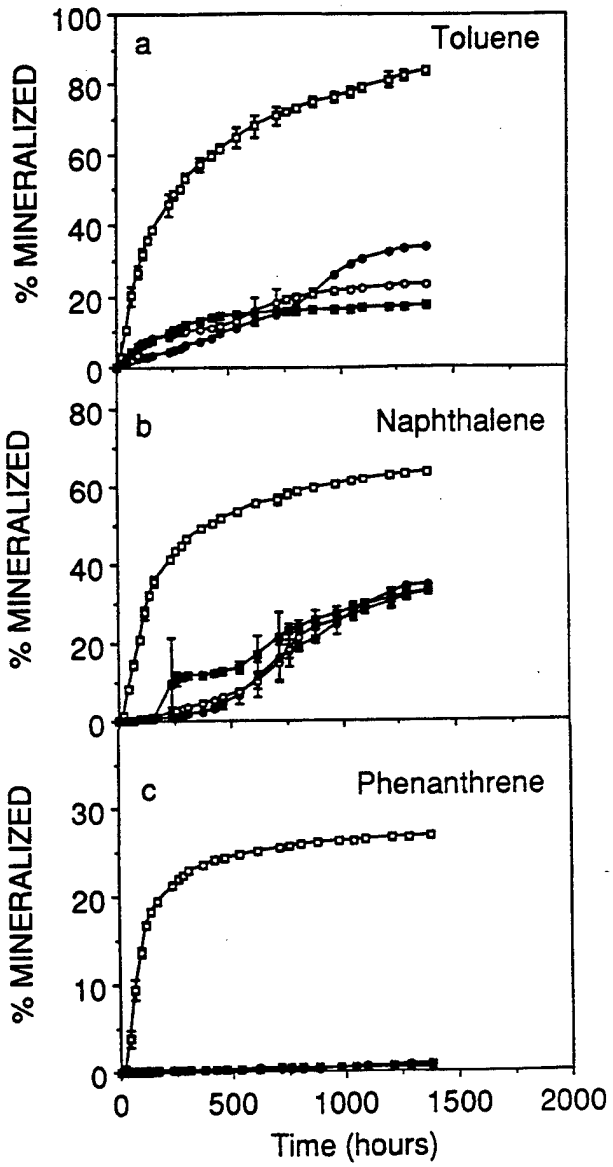

Figure 2. Time courses for the mineralization of toluene (a, 5 μg mL^{-1}), naphthalene (b, 1 μg mL^{-1}), and phenanthrene (c, 0.5 μg mL^{-1}) in Eielson soil slurries treated with HDTMA at 0 (controls, open squares), 10% (open circles), 30% (solid squares) or 70% (solid circles) of the cation-exchange capacity.

The toxicity of HDTMA toward Gram-negative bacteria was investigated further since these organisms are well-known as xenobiotic degraders. *P. putida* strain 17484 (a naphthalene-degrading bacterium), which showed the highest sensitivity to HDTMA (LC_{50} = 4 μM), was rapidly killed by the unbound cation. When exposed to 10 μM HDTMA, over 90% of cells were killed within 30 min. After a 1-h exposure, fewer than 2% of the cells remained viable. When smectite clay (CEC = 90 mequiv/100 g) was added to 100 μM HDTMA solutions, survival of the pseudomonad increased in inverse proportion to the calculated (based on sorption isotherm data) concentration of unbound cation. Figure 3 shows that as little as 1.2 mg of smectite clay could completely alleviate the toxicity of a 100 μM HDTMA solution (2 mL) and that the 1-h survival rates were markedly improved in the presence of smectite even when the calculated aqueous HDTMA concentration was above 10 μM.

Table 2. Characteristics and Identification of Bacteria Isolated from HDTMA-Treated Marlette A Horizon Soil and HDTMA LC_{50} Values for Soil Isolates and Several Type Strains

organism	gram stain	spore formation	motility	colony morphology	cell morphology	identification[a] FAME[b]	identification[a] Biolog	HDTMA LC_{50} (μM)
soil isolates								
A	+	+	–	rough, tan	large rods, pairs	*B. megaterium* (0.688)	*Coryn. jeikeium* (0.539)	44
C	+	+	–	rough, white	large rods, tetrads	*B. megaterium* (0.716)	*B. insolitus* (0.50)	46
D	+	+	–	sprawling, tan	large rods, chains	*B. megaterium* (0.541)	*B. insolitus* (0.548)	514
F	+	+	–	round, brown	large rods, pairs	*B. megaterium* (0.733)	*B. insolitus* (0.580)	61
H	+	+	–	rough, white	large rods, single	*B. mycoides* (0.156)	no match	54
J	–	–	+	round, red	small rods, single	nd[c]	nd	7
O	+	+	+	rough, white	large rods, chains	*B. gordonae* (0.592)	*C. pseudodiphtheria* (0.509)	46
P	±	–	+	opaque, slimey	small rods	*Enterobacter* sp. (0.420)	*Enterobacter* sp. (0.64)	51
W	+	–	+	round, orange	large rods, single	no match	no match	51
X	+	–	–	round, yellow	small cocci	*Micrococcus luteus* (0.71)	*B. insolitus* (0.814)	28
Y	+	+	+	round, yellow	rods, single	*B. filicolonicus* (0.287)	*B. megaterium* (0.577)	28
type strains								
P. putida								4
M. luteus								53
R. rhodochrous								37
A. globiformis								7
Alcaligenes sp. (strain NP-Alk)								8

[a] Primary, library-based matches are given with similarity coefficients in parentheses. [b] Identifications based on Fatty Acid Methyl Ester analysis. [c] Not determined due to poor growth on pretest medium.

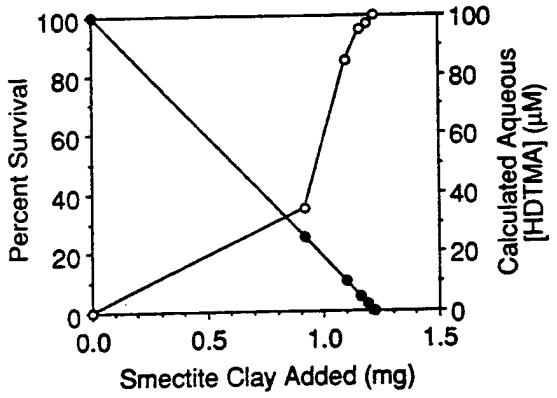

Figure 3. Influence of smectite clay additions on the survival of *P. putida* (open circles) in 100 μM HDTMA solutions (2 mL) following a 1-h treatment. The calculated equilibrium aqueous-phase HDTMA concentrations (solid circles) are shown on the right-hand ordinate.

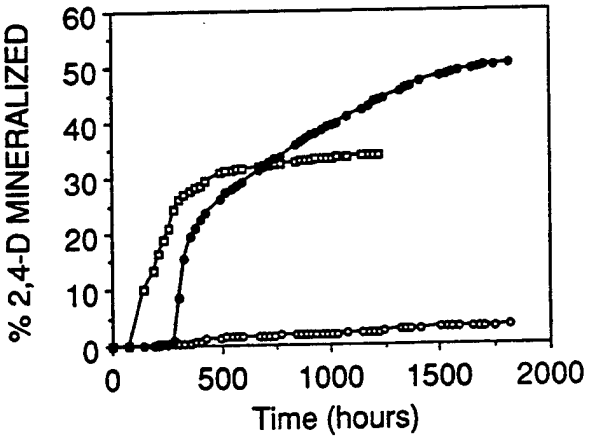

Figure 4. Time courses for the mineralization of 2,4-D (10 μg mL^{-1}) in Marlette A soil slurries without the addition of HDTMA (open squares), with the addition of unbound HDTMA at 70% of the CEC (open circles), or with the addition of an equivalent amount of HDTMA prebound to sterile Marlette B_t soil (solid circles).

When HDTMA was presented in a prebound form in substrate mineralization assays, similar reductions in toxicity and improvements in mineralization efficiencies were noted (Figure 4). Addition of free HDTMA resulted in nearly complete inhibition of 2,4-D mineralization (see also Figure 1), with less than 5% of the substrate mineralized after 1800 h. In contrast, addition of prebound HDTMA delayed the onset of mineralization but allowed extensive mineralization of the substrate.

Similar results were obtained in naphthalene mineralization experiments using the Eielson aquifer sediment. Figure 5 shows that chemical or biological scavenging of unbound HDTMA completely alleviated the toxicity of the QAC with nearly coincident mineralization curves for the clay- and bacteria-treated samples with the untreated (no HDTMA) control. The treated control, in which residual HDTMA was not scavenged, showed a longer lag period and a reduction in the extent of naphthalene mineralization. In bacteria-treated controls containing [^{14}C]HDTMA and unlabeled naphthalene, 36% of the label was recovered as CO_2 over the second (3-day) equilibration period, indicating that a significant fraction of the added HDTMA remained in a biologically available form following the initial (1-day) equilibration period.

Other QACs, which are also effective in forming highly sorptive phases when exchanged on clay mineral surfaces, were tested for their toxicity toward the HDTMA-sensitive organism, *P. putida*. Figure 6 shows that the C-16 cations, HDTMA and CPB were more than twice as toxic as other

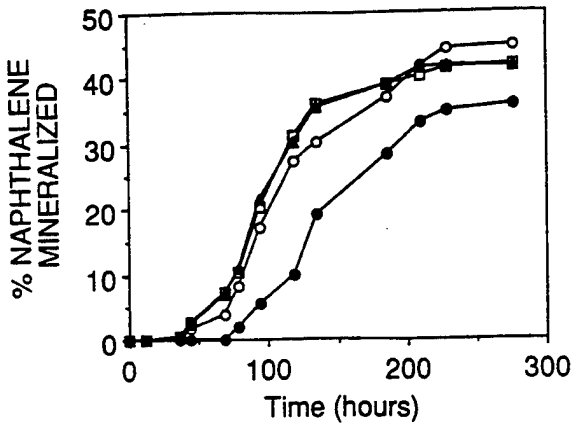

Figure 5. Time courses for the mineralization of naphthalene. Eielson soil was treated with HDTMA at 50% of the CEC, followed by additions of smectite clay (open circles), a suspension of HDTMA-degrading bacteria (solid triangles), or no additions (treated controls, solid circles) before inoculation with 1 g of active Eielson soil (time zero). Data for an untreated control (naphthalene but no HDTMA, open squares) is also shown.

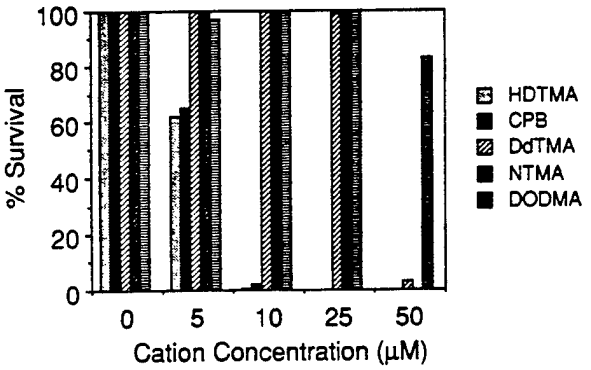

Figure 6. Relative toxicities of five quaternary ammonium compounds to *P. putida* assayed as the percent survival following a 1-h treatment at various cation concentrations.

monoalkyl cations (NTMA and DdTMA) with shorter alkyl chains (C-9 and C-12, respectively). The dialkyl (C-18) cation, DODMA, exhibited the lowest toxicity and was ~10 times less toxic than HDTMA (over 80% survival at a concentration of 50 μM as compared to 65% survival at 5 μM HDTMA).

Figure 7 shows that compared to untreated controls, DODMA exerted little toxicity toward the degradative microbial community in this soil. Except for minor decreases in the rate and extent of salicylate and naphthalene mineralization, treated samples behaved much like the untreated controls.

Discussion

In this paper, we have attempted to assess the effect of QACs on the heterotrophic activities of soil microbial communities. Addition of HDTMA to soil caused inhibition of heterotrophic activity and toxicity to the microbial populations. Results from two soils employing several "natural" and "xenobiotic" organic compounds illustrate these findings. In the Marlette A horizon soil, HDTMA had little adverse effect on the mineralization of glucose, but inhibited the mineralization of the more refractory compounds, salicylate and 2,4-D. With all compounds, lag periods preceding rapid mineralization increased with

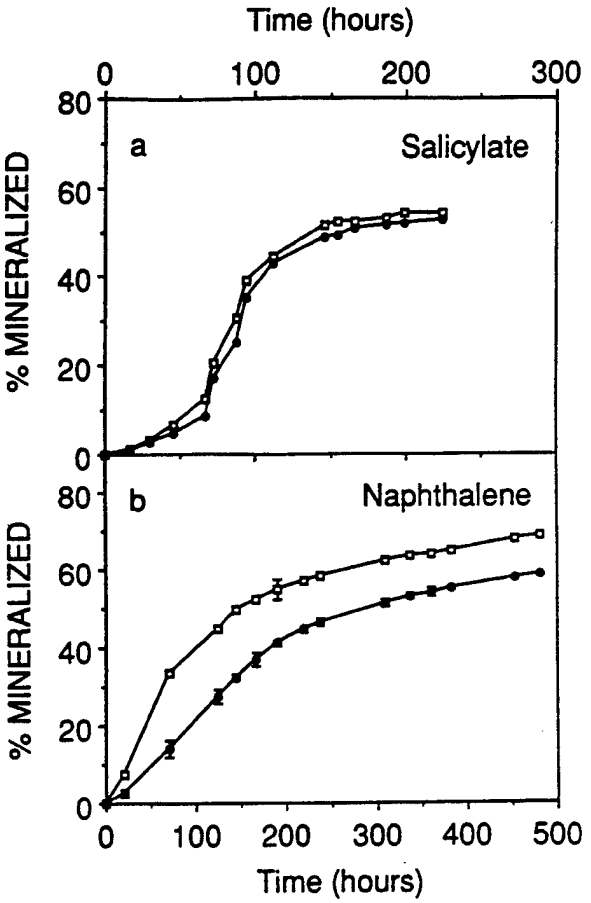

Figure 7. Time courses for the mineralization of salicylate (a, 5 μg mL^{-1}) and naphthalene (b, 1 μg mL^{-1}) in Eielson soil slurries treated with DODMA at 0 (controls, open squares) or 50% (solid circles) of the soil cation-exchange capacity. Note different time scales for panels a and b.

increasing HDTMA treatment level as the affected degradative populations became reestablished. Such recovery did not occur in the case of 2,4-D mineralization at the 70% treatment level during an 800-h incubation. In the Eielson aquifer sediment, the microbial community was well-adapted for mineralization of the aromatic hydrocarbons, toluene, naphthalene, and phenanthrene, as evidenced by the steep initial slopes in the mineralization curves for these compounds in untreated controls. Even at the 10% treatment level, however, increased lag periods and reduced extents of mineralization were noted for all compounds, with the magnitude of the effect increasing with increased complexity and decreased solubility of the substrate.

Assuming that the length of the lag periods preceding mineralization in untreated controls is an indication of the size of the indigenous degradative population, the Marlette soil contains fewer 2,4-D degrading organisms than salicylate- or glucose-degrading organisms. If HDTMA treatment kills a constant percentage of the degradative populations, too few organisms may survive treatment to permit their reestablishment in tests with complex substrates. Alternatively, HDTMA may be selectively toxic toward organisms able to degrade more complex substrates. The frequent enrichment of *Pseudomonas* spp., *Alcaligenes* spp., and *Arthrobacter* spp. in degradation experiments with 2,4-D (*30-33*) and aromatic substrates (*28, 34-40*) and the apparent sensitivity of these genera to HDTMA (Table 2) support this explanation.

A third possibility is that the addition of organic cations to soils in quantities capable of satisfying a significant fraction of the CEC limits the availability of the target compounds by increasing the sorptive capacity of the soils. Adapting the biphasic sorption equation of Boyd and Sun (*41*) for calculation of equilibrium aqueous-phase solute concentrations in systems with two distinct sorptive phases [with partition coefficients for solutes binding to HDTMA phases (K_{HDTMA}'s) = K_{ow}'s for the solutes (*3*) and using published K_{oc} and K_{ow} values (*42*)], the reductions in aqueous-phase concentrations of toluene, naphthalene, and phenanthrene in Eielson soil due to treatment with HDTMA (50% of CEC) would be 4.18–2.25 $\mu g\ mL^{-1}$, 517–141 ng mL^{-1}, and 50–5 ng mL^{-1}, respectively. Similar reductions might explain the minor decreases in the initial rate and extent of naphthalene mineralization observed in the Eielson sediment treated with the relatively nontoxic cation, DODMA, at 50% of the CEC (Figure 7). Reduced dissolved solute concentrations are unlikely, however, to be responsible for the extended acclimation periods (Figure 1) and poor mineralization efficiencies (Figure 2) observed in HDTMA treated soils. Thus, the absence of phenanthrene degraders in HDTMA-treated Eielson aquifer sediment (Figure 2c) is likely the result of toxicity to a highly sensitive and/or small degradative community and not to sorption-limited growth or activity of the organisms. The toxic effects of HDTMA were confirmed by the dramatic decreases in the numbers and diversity of bacteria recovered from soils treated wtih HDTMA (50% CEC).

In pure culture, bacteria were extremely sensitive to dissolved HDTMA with LC_{50} values ranging from 4 to 514 μM. All the Gram-positive isolates tested (with the exception of the type strain of *A. globiformis*) gave LC_{50} values at least 3-fold higher than the Gram-negative isolates (with the exception of the slime-producing isolate P). Salt and Wiseman (*20*) reported that Langmuir isotherms are characteristic for HDTMA binding to bacteria and that the HDTMA concentration corresponding to the asymptotic binding capacity approximated the threshold concentration at which cell leakage and death occurred. Reversals in the electrophoretic mobilities of bacterial cells also occurred at HDTMA concentrations above strain-specific thresholds (*20*). The threshold concentration was 4-fold higher for *B. megaterium* than for *E. coli*, in general agreement with the LC_{50} relationships observed here for *Bacillus* spp. and Gram-negative organisms (Table 2).

With *P. putida*, HDTMA toxicity was reduced when 100 μM solutions were titrated with the clay mineral, smectite, prior to toxicity assays, indicating that the cation was only active in its unbound form. Toxicity was eliminated when the cation-exchange capacity provided by the clay equaled the number of equivalents of HDTMA present. HDTMA added to soils at concentrations below the CEC should be predominantly in the bound state at equilibrium. However, initial concentrations in added stock solutions were always greater than 1 mM. At 100% of the CEC, HDTMA concentrations in PBS solutions added to the Marlette A, Marlette B_t, and Eielson soils would be 23, 25.6, and 16 mM, respectively. Although the exchange of HDTMA to clay minerals and soils is an extremely rapid and highly selective process, unprotected bacteria would compete as exchange and/or sorptive sites initially. Time course studies showed that *P. putida* was rapidly deactivated by free HDTMA at very low (10 μM) concentrations. Furthermore, even after a 1-day equilibration of ^{14}C-labeled HDTMA (at 50% of the CEC) with the Eielson aquifer material, 36% of the label was recovered as CO_2 following inoculation with an HDTMA-degrading bacterium. This suggests that a significant fraction of the cation remains in an available form for several hours after addition to soil. Initially, a portion of the HDTMA molecules may bind to soils in a "tail-to-tail" configuration with van der Waals binding of one alkyl chain to the alkyl chain of a second molecule anchored by cation exchange to a mineral surface. Toxicity (and availabililty) may therefore persist until the cations become immobilized at exchange sites.

Addition of prebound HDTMA to active soils, reinoculation of sterile HDTMA-treated soils with fresh soil, or scavenging of residual unbound HDTMA by organisms able to utilize it as a sole carbon and energy source alleviated toxicity and permitted degradation of the test solutes by indigenous microorganisms. In open systems, such as aquifers and subsoils, bacterial migration would serve to recolonize HDTMA-treated soils once the initial toxicity of the QAC was attenuated as it became bound to soil clays. Other QACs, such as DODMA, are also effective in forming highly sorptive phases for organic contaminants when exchanged on soil minerals and are significantly less toxic than HDTMA.

The overall effects of treating soil and aquifer materials with HDTMA will probably occur in two stages. The addition of HDTMA to soil initially exerts an adverse impact on the activities of aerobic heterotrophic, contaminant-degrading bacteria. In our closed experimental systems, this impact was manifested by increased lag periods before degradation commenced and slower overall degradation rates. However, once HDTMA became bound to cation-exchange sites in soils, its toxicity was greatly diminished. While the specific susceptibilities of anaerobic organisms (important in the subsurface degradation of certain contaminants) to QACs may vary, the sparing effects of QAC adsorption would likely be similar for these organisms. This suggests that in field applications, the treated zone may be repopulated by degradative bacteria once HDTMA becomes bound via cation-exchange reactions, and residual, unbound cation is itself degraded or washed out of the treated zone. Other QACs, such as DODMA, are inherently less toxic to soil bacteria than HDTMA and may enhance the compatibility of chemical modification and biodegradation.

Acknowledgments

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APPENDIX C

Bioavailability of Naphthalene Sorbed to Cationic Surfactant-Modified Smectite Clay

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Abstract

The bioavailability of naphthalene sorbed to hexadecyltrimethylammonium (HDTMA)-modified smectite clay was evaluated by modeling naphthalene mineralization kinetics in dilute clay slurries and in clay-free controls. Sorbed naphthalene was directly available to *Pseudomonas putida* strain 17484, as evidenced by initial rates and extents of naphthalene mineralization that significantly exceeded predicted values assuming sorbed naphthalene was unavailable. For the soil isolate, *Alcaligenes* sp. strain NP-Alk, sorbed naphthalene was unavailable and measured rates agreed closely with predicted rates. For this bacterium, sorbed naphthalene was available only upon its desorption from the HDTMA-modified smectite. This desorption was very rapid from unaggregated HDTMA-smectites and from HDTMA-clay aggregates of less than 0.25-mm diameter. Naphthalene mineralization in the presence of larger clay aggregates (0.25 to 1-mm diameter) was desorption rate-limited. Contaminants sorbed to HDTMA-modified soils or clays should be largely bioavailable to bacteria, since the desorption rates from these materials are high and some degradative bacteria have the ability to directly utilize the sorbed contaminants.

Introduction

Enhanced immobilization of common organic groundwater contaminants in soils and subsoils can be achieved by modifying these materials with cationic surfactants (1-3). Quaternary

ammonium cations of the form $[(CH_3)_3NR]^+$, where R is a large ($>C_{10}$) alkyl hydrocarbon, readily replace native inorganic cations on the exchange sites of soil clays (4), resulting in the formation of effective sorptive phases for nonionic organic contaminants (NOCs). In such surfactant-modified subsoils, the soil-water distribution coefficients for NOCs, such as ethylbenzene (2) and tetrachloroethylene (1, 2), increased by more than 100 times over those observed in native B horizon soils. These results suggest that aquifer materials or subsoils could be modified *in situ* via injections of cationic surfactants to create sorptive zones that could intercept and immobilize advancing contaminant plumes. The feasibility of this approach has been demonstrated in recent experiments by Burris and Antworth (5). Although this technology is potentially very useful for managing contaminant plumes by minimizing further contamination of the aquifer materials and reducing down gradient contaminant concentrations in groundwater, it does not permanently remove contaminants from the environment. Coupling contaminant immobilization with *in situ* biodegradation would provide a comprehensive soil restoration technology to effectively eliminate target contaminants (3, 6). The bioavailability of NOCs sorbed to surfactant-modified soils and clays is a critical aspect of this proposed technology.

The influence of sorption on the biodegradation of organic contaminants has been recognized as an important, albeit, poorly understood, issue in bioremediation (7-19). Factors such as the

compound's chemical structure, nature of the sorbent, the residence time of the sorbed compound, and the desorption rate may influence the biodegradation of sorbed compounds. The fractions of 2,4-dichlorophenoxy acetic acid (2,4-D) (8) and polyaromatic hydrocarbons (PAHs) (9) sorbed to soil, and diquat (10) sorbed to clay were completely unavailable for degradation. In contrast, toluene (11), PAHs (9), polychlorinated dibenzo-p-dioxins (PCDDs) (12), naphthalene (13), 2,4-D (14) and phenol (15) sorbed to soils and sediments, and benzylamine (16), and phenol (17) sorbed to clays were available for biodegradation, probably following desorption into the aqueous phase. Differences in the organic carbon (OC) contents between two soils, and the duration of soil-PAH contact were postulated to explain why PAHs were degraded in one soil (1% OC) and not another soil (13.6% OC) (9).

The bioavailability of sorbed compounds may also be affected by the microorganisms themselves. Guerin and Boyd (18, 19) recently used a kinetic method to show that the ability to directly utilize soil-sorbed naphthalene is a species-specific characteristic. *Pseudomonas putida* strain 17484 was able to directly access labile sorbed naphthalene and to promote the desorption of non-labile naphthalene from the interior of soil particles. In contrast to strain 17484, *Alcaligenes* sp. strain NP-Alk utilized only aqueous phase naphthalene, and most of the soil-sorbed fraction remained unavailable. In this study we examine the bioavailability of naphthalene sorbed to a model

sorbent, hexadecyltrimethylammonium (HDTMA)-modified smectite, using the same two organisms. Our aim was to evaluate the coupled immobilization-biodegradation technology proposed and to gain further insights to the sorption-desorption behavior of NOCs with the modified smectite.

Materials and Methods

Preparation of HDTMA modified-Smectite. Smectite (Wyoming bentonite) with a cation exchange capacity (CEC) of 90 meq (100 g)$^{-1}$ was obtained from the American Colloid Co. (Arlington Heights, IL). The smectite was dispersed in distilled water (10 g l^{-1}) and the clay size fraction was separated by gravity sedimentation.

HDTMA-smectite was prepared by adding an aqueous solution of HDTMA bromide (Sigma Chemical Co., St. Louis, MO) to the stirred clay suspension (of known clay concentration) in the amount required to achieve 50% saturation of the clay's CEC (referred to as 50% HDTMA-smectite). At 50% saturation, all the HDTMA is irreversibly bound by cation exchange and not by hydrophobic bonding which tends to be reversible(4). The 50% HDTMA-smectite suspension was equilibrated at 23 °C with stirring for approximately 16 h. The suspension was centrifuged at 10140 × *g* for 10 min at 20 °C in a Sorvall RC5-C centrifuge, resuspended in distilled water, and re-centrifuged. This washing procedure was repeated 3 times. The final pellet was frozen in a dry-ice-acetone bath, lyophilized and crushed to a fine powder with a

mortar and pestle. This material will be referred to as unaggregated HDTMA-smectite.

Two different particle size fractions of 50% HDTMA-smectite were also prepared as described above, with the following modifications. The washed clay preparation was dewatered by blotting the suspension onto Whatman No. 1 filter paper. The resultant clay paste was transferred to watch glasses and oven-dried at 110 °C. The HDTMA-smectite dried into thick, hard cakes that were ground in a mortar and pestle. The ground clay was passed through nested sieves (10, 18 and 60 mesh) to obtain two aggregate size fractions: less than 0.25 mm, and 0.25 to 1 mm diameter. The organic carbon contents of the 50% HDTMA-smectite preparations were determined using a Dohrmann model DC190 carbon analyzer with a model 183 boat sampling module (Table 1). The oven-dried clays did not disaggregate upon wetting.

Naphthalene sorption isotherms. Sorption isotherms of ^{14}C-naphthalene on 50% HDTMA-smectite were performed by the batch method as described previously (1). Linear regressions of plots of equilibrium sorbed concentrations versus equilibrium aqueous concentrations were used to obtain the naphthalene partition coefficient, K_p (Table 1).

Bacterial cultures. The naphthalene-degrading isolates, *Pseudomonas putida* (ATCC strain 17484) and an *Alcaligenes* sp. (strain NP-Alk), isolated from petroleum-contaminated soil (18), were used in these studies. The bacteria were cultured in high buffer broth (18) plus naphthalene (final concentration, 200 mg

l^{-1}) at 23 °C with shaking at 200 rpm. The bacteria were grown to early stationary phase, harvested by centrifugation (12100 × *g*, 10 min), and washed once in phosphate-buffered saline (PBS) (19).

[^{14}C]Naphthalene mineralization assays. The mineralization of ^{14}C-labeled naphthalene (8.9-10.3 mCi $mmol^{-1}$, 98% radiochemical purity; Sigma) in the presence or absence of 50% HDTMA modified-smectite was determined according to the method of Guerin and Boyd (18). In summary, sterile 50% HDTMA-smectite was equilibrated in 75 ml of PBS containing total naphthalene concentrations of 60 or 100 ng ml^{-1} and a total activity of approximately 3500 dpm ml^{-1} in 155 ml serum vials crimp-sealed with teflon-lined septa. Clay to solution ratios were varied to produce a range of equilibrium aqueous phase naphthalene concentrations from 6 to 95 ng ml^{-1}. These ratios and concentrations were calculated based on the naphthalene partition coefficient for each HDTMA-smectite preparation. Clay-free controls contained only aqueous phase naphthalene at concentrations ranging from 20 to 100 ng ml^{-1}.

Following equilibration, vials were inoculated with 0.75 to 1 ml of the washed cell suspensions to a final cell density of approximately 1×10^6 to 1×10^7 cells ml^{-1}. The vials were incubated at 23°C with shaking at 200 rpm to ensure complete mixing of the clay and cells. At predetermined times, combined aqueous and gaseous subsamples were removed from the vials by syringe and transferred to sealed test tubes containing 1 ml of 2N HCl. The (evolved) $^{14}CO_2$ was collected on fluted filter paper

saturated with 2N-KOH and placed within a plastic cup suspended from the tube stopper. After 18 to 24 h, the filter papers were removed and transferred to glass scintillation vials containing 7.5 ml of Safety-Solve scintillation fluid along with 2 ml of 95% ethanol, which was used to rinse the plastic cup. The activity of the $^{14}CO_2$ produced was measured by liquid scintillation counting (Packard 1500 Tri-Carb Liquid Scintillation Analyzer; Downers Grove, IL) with external standard quench correction. Mineralization data are presented as a percentage of the total initial activity converted to $^{14}CO_2$ as a function of time.

Theory. The percent (P) of the naphthalene mineralized as a function of time (t, in minutes) was fitted to a three-parameter, coupled degradation-desorption model(18) of the form:

$$P = v_2 t + ((v_1 + v_2)(1-e^{-kt}))/k.$$

This model was shown previously (18) to provide excellent fits to data in which an initial first-order phase of mineralization of dissolved and rapidly desorbed naphthalene is followed by a linear (zero-order) phase of mineralization of more slowly desorbing naphthalene. The model provides estimates of the progress of the first-order phase of the reaction, v_1 (% min^{-1}), and the first-order mineralization rate constant, k (min^{-1}). The initial mineralization rate ($v_1/100$ times the initial naphthalene concentration; ng ml^{-1} min^{-1}) and the extent of naphthalene mineralization (v_1/k; %) during the initial phase of the reaction

are derived from these estimates. In addition, the model estimates a zero-order naphthalene mineralization rate (v_2; % min^{-1}) from which a pseudo first-order desorption rate constant can be derived. This linear phase is only observed when desorption rates are intermediate and of the same order as biodegradation rates. When desorption rates are much faster than, or much slower than biodegradation rates, this linear mineralization phase is absent, v_2 is equal to zero, and the model degenerates to a simple first-order expression (18).

Since the initial naphthalene concentrations used in this study were approximately $0.5(K_M)$ for naphthalene utilization by the organisms (18), mineralization rates were directly proportional to naphthalene concentrations in sorbent-free controls (18). *Assuming sorbed naphthalene is unavailable* for microbial degradation, reductions in aqueous phase naphthalene concentrations due to sorption should cause concomitant reductions in mineralization rates. These rates can be predicted based on the equilibrium aqueous phase naphthalene concentrations, calculated from sorption isotherms, and the linear relationship between mineralization rate and naphthalene concentration established in sorbent-free controls. Deviations of measured from predicted rates can then be interpreted in terms of the relative bioavailability of naphthalene in different sorbent systems. In an analogous way plots of the extent of mineralization (v_1/k) data versus the equilibrium aqueous phase naphthalene concentration can provide information about the

availability and rate of desorption of bound naphthalene. If sorbed naphthalene is unavailable and if desorption is slow relative to biodegradation, a linear decrease in the percentage of the total naphthalene mineralized should be evident as the clay to solution ratio increases and the equilibrium aqueous phase naphthalene concentration decreases. A more complete description of the approach used to assess bioavailability of sorbed substrates can be found in Guerin and Boyd (18, 19).

Determination of HDTMA-Smectite Toxicity. To determine whether the 50% HDTMA-smectite was inhibitory to strains 17484 and NP-Alk, amounts of 50% HDTMA-smectite, approximately equal to those used in bioavailability experiments were resuspended in 75 ml of PBS and shaken overnight. HDTMA-smectite preparations and negative (without HDTMA-clay) control vials were inoculated with approximately 1×10^7 cells ml^{-1} from a washed suspension of strain 17484 (see above) and shaken for 6 h. Immediately following inoculation and at various intervals, the cultures were serially diluted in PBS containing 3% Tween 80, to inactivate the HDTMA (21). Aliquots of the appropriate dilutions were then spread over the surface of nutrient agar plates (Difco). Viable plate counts were determined following incubation at 23°C for 2 to 3 d. An additional set of positive controls containing PBS and dissolved HDTMA in an amount equal to that exchanged onto the clay was included to confirm that clay bound HDTMA is significantly less toxic to bacteria than free HDTMA.

Results

Because aqueous phase HDTMA is toxic to bacteria at concentrations as low as 10 μM (22) the effect of the 50% HDTMA-smectite on the survival of *P. putida* 17484 was investigated. This test was not repeated for strain NP-Alk, because strain 17484 is more sensitive to HDTMA than strain NP-Alk (22). Viable cell counts for strain 17484 were not reduced by exposure of strain 17484 to 10 mg ml^{-1} of 50% HDTMA-smectite, a concentration routinely used in the bioavailability assays, over a 6 h incubation period (data not shown). The lack of inhibitory effects of the 50% HDTMA-smectite on the survival of strain 17484, confirmed that the cation was irreversibly bound to the clay .

The bioavailability of naphthalene sorbed to the 50% HDTMA-smectite was examined by comparing the rates and extents of naphthalene mineralization in clay-free and clay-containing systems. Representative naphthalene mineralization time courses by *P. putida* strain 17484 at various clay to solution ratios are shown in Figure 1a. The mineralization of naphthalene by strain 17484 was rapid in all cases with sorption only slightly affecting naphthalene mineralization compared to the clay-free control. Mineralization curves at all clay to solution ratios were essentially first order, with little evidence of a desorption-limited, linear phase of mineralization. These data indicate that desorption was very rapid in these systems.

For *P. putida* 17484, initial naphthalene mineralization

rates in the presence of 50% HDTMA-smectite were greater than the rates predicted on the basis of the equilibrium aqueous phase naphthalene concentrations, as indicated by their position above the clay-free control line (Figure 1b). To support such high initial rates, strain 17484 had immediate access to a portion of the sorbed naphthalene in addition to that present in the aqueous phase at equilibrium, since even rapidly desorbing naphthalene would not alter initial mineralization rates. The high extents of naphthalene mineralization (v_1/k values) by strain 17484 in clay-containing systems was consistent with rate data indicating the availability of sorbed naphthalene to this organism (Figure 1c). With strain 17484, both the initial rate and the extent of mineralization tended to increase as the clay to solution ratio increased.

In contrast to strain 17484, increasing clay to solution ratios caused progressive reductions in the initial slopes of naphthalene mineralization curves for strain NP-Alk (Figure 2a). Again, there was little evidence of desorption-supported mineralization during the latter part of the experiments.

Initial naphthalene mineralization rates by strain NP-Alk in the presence of 50% HDTMA-smectite were very close to predicted rates, falling near the clay-free control line (Figure 2b). This indicated that the clay-sorbed naphthalene was essentially unavailable to strain NP-Alk, and that initial mineralization rates were dictated by the solution-phase naphthalene concentrations. The extents of naphthalene mineralization (v_1/k

values) by strain NP-Alk during the initial first-order phase of the time course significantly exceeded the values predicted if sorbed naphthalene were both unavailable and slowly desorbing (Figure 2c). These results indicated that rapid desorption of naphthalene accompanied the degradation of aqueous phase naphthalene. This desorbed naphthalene became available to strain NP-Alk during the initial phase of the incubation and was not followed by a desorption-limited, zero-order phase of mineralization.

Using a gas-purge apparatus, we had previously observed that the desorption rate of alkylbenzenes sorbed to HDTMA-clay aggregates was dependent on aggregate size (23). To examine the effect of the naphthalene desorption rate on mineralization kinetics by strain NP-Alk, we conducted bioavailability assays using 50% HDTMA-clay aggregates of different sizes as sorbents.

As with non-aggregated HDTMA-smectite, naphthalene mineralization curves in the presence of the small clay aggregates appeared to follow simple first order kinetics (Figure 3a) with initial mineralization rates decreasing as the aggregate to solution ratio increased. The asymptotes of the mineralization curves remained horizontal for many hours at values significantly below the control curve indicating a very slowly desorbing fraction of sorbed naphthalene in these aggregates. In contrast, sorption of naphthalene to the larger (0.25 to 1-mm) clay aggregates caused mineralization curves to deviate from simple first order kinetics (Figure 3b). Sorption-mediated reductions in

dissolved naphthalene concentrations caused reductions in initial mineralization rates and decreases in the extent of naphthalene mineralized during the first 500 minutes of the assays. Over the remainder of the time course, a slower linear phase of mineralization ensued, although a significant fraction of the sorbed naphthalene still remained unavailable. The coupled degradation-desorption model gave an accurate description of naphthalene mineralization kinetics by strain NP-Alk in the presence of the large clay aggregates, with significant reductions (F test at 0.05 significance level) in the residual sums of error compared with those obtained using a simple (two-parameter) first order model.

Initial mineralization rates in slurries containing either clay size fraction decreased with decreasing equilibrium aqueous phase naphthalene concentrations indicating that naphthalene sorbed to HDTMA-clay aggregates was unavailable to this organism (Figure 4a). Rapid desorption of a fraction of the sorbed naphthalene from the small aggregates gave rise to v_1/k values which, with one exception, exceeded the predicted values. All v_1/k values were less than the clay-free control value (Figure 4b) indicating that a portion of the sorbed naphthalene remained resistant to desorption.

In samples containing the larger clay fraction, the extents of naphthalene mineralization also decreased as the clay to solution ratio increased (Figure 4b), with a larger fraction of the sorbed naphthalene residing in a slowly desorbing or non-

labile state. In the larger clay aggregates, only a relatively small portion of the clay-sorbed naphthalene desorbed quickly during the first-order phase of mineralization, as indicated by v_1/k values which only slightly exceeded the expected values (Figure 4b). In experiments with large clay aggregates, the coupled degradation-desorption model yielded positive zero-order mineralization rates, with v_2 values ranging from 0.003 to 0.006% min^{-1}. Normalization of v_2 to the fraction of naphthalene initially sorbed produced pseudo first-order desorption rate constants of 0.005 to 0.012 h^{-1}.

Discussion

Naphthalene bioavailability assays in the presence of HDTMA-modified smectite clay produced significantly different results for the two bacterial species, *P. putida* strain 17484 and *Alcaligenes* sp. strain NP-Alk. These differences relate to the relative ability of each strain to directly utilize and to promote desorption of the clay-sorbed naphthalene. *P. putida* strain 17484 appeared to have direct and immediate access to a fraction of the sorbed naphthalene, probably that which is localized near the surface of the modified-smectite. Utilization of this material may promote the desorption of additional naphthalene located more deeply within the clay tactoid by establishing steep intraparticle concentration gradients. By comparison, clay-sorbed naphthalene was essentially unavailable to strain NP-Alk which relies on the passive diffusion of the

sorbed naphthalene into the aqueous phase as a prerequisite for utilization.

The differential bioavailability of clay-sorbed naphthalene to *P. putida* 17484 and strain NP-Alk observed in this study was consistent with previous results in soil slurries (18, 19). Due to much slower naphthalene desorption kinetics from soil compared to unaggregated 50% HDTMA-smectite, however, utilization of soil-sorbed naphthalene by strain 17484 was desorption-limited and showed biphasic kinetics (18), rather than the simple first order kinetics observed in these experiments with unaggregated HDTMA-smectite. Even in soil studies, however, the ability of strain 17484 to promote desorption of bound naphthalene was indicated by pseudo first order desorption rate constants which averaged 0.047 h^{-1}, and the rapid and complete mineralization of the sorbed naphthalene. Increased desorption rates of NOCs from HDTMA-modified soils and clays compared to natural soils have also been observed in column transport experiments (5) and using a gas purge technique (23).

Strain NP-Alk has consistently demonstrated an inability to utilize or promote the desorption of naphthalene associated with a variety of natural and synthetic sorbents (18, 19, 24). For naphthalene sorbed to large clay aggregates, pseudo first order desorption rate constants averaged between 0.005 and 0.012 h^{-1}, about the same as values calculated previously for soil-sorbed naphthalene (0.007 h^{-1}; 18). With this organism, a large fraction of the naphthalene sorbed to large clay aggregates remained

unavailable for mineralization, as in our ealier soil studies (18). As HDTMA-clay aggregate size decreased, the rate and extent of desorption and mineralization increased as indicated by changes in the shapes of the mineralization curves and increases in v_1/k values in progressing from large aggregates to small aggregates to unaggregated clay (Figures 2c and 4b). In a similar study (17), the biodegradation kinetics of phenol sorbed to clay aggregates was influenced by the size of the aggregates. Varying diffusion path lengths of phenol out of clay aggregates of different sizes was the major factor affecting the shape of the mineralization curve and persistence of the phenol (17).

In gas purge studies, the desorption rate of *n*-propylbenzene from the <0.25-mm diameter HDTMA-modified smectite fraction was ten times greater than that from the 0.25 to 1-mm diameter fraction (23). Desorption of alkylbenzenes from the small clay aggregates was sufficiently rapid so that gas purge curves could be fit to an equilibrium (2-box) model, suggesting that all of the alkylbenzene sorbed within the aggregates was in equilibrium with the solution phase (referred to as a labile sorbed phase). In contrast, the purge curves from the 0.25 to 1-mm clay aggregates were best fit to a bicontinuum (3-box) model indicating the presence of regions within the aggregates from which alkylbenzenes desorbed more slowly (referred to as a non-labile sorbed phase) (23). The slower alkylbenzene or naphthalene desorption rate with the larger clay aggregates is consistent with a mechanism whose rate-limiting step is solute

diffusion within the aggregate; the longer diffusion path lengths in the larger clay aggregates leading to lower desorption rates. The aggregate size-dependent kinetics of naphthalene mineralization indicated by our bioavailability assays are consistent with these physical measurements of desorption kinetics utilizing the gas purge apparatus (23).

Despite more rapid desorption kinetics of naphthalene associated with HDTMA-clay as opposed to natural soil organic matter, differences in the efficiency of sorbed naphthalene utilization by the two organisms were still evident. The ability of strain 17484 to utilize surface-localized sorbed naphthalene suggests that this organism may associate more intimately and/or extensively with the sorbent surface than strain NP-Alk. The greater hydrophobicity and propensity of strain 17484 to attach in sand columns (24) are consistent with this interpretation.

In environmental applications, the bioavailability of NOCs sorbed to soils or clays modified with quaternary ammonium cations such as HDTMA, should be high due to relatively rapid contaminant desorption from these materials. Once toxic effects are alleviated through cation binding, bacteria could attach directly to the treated solids and promote this desorption. Contaminant sorption is therefore not expected to prevent the biodegradation of organic contaminants in these systems.

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List of Figures

Figure 1. a: Naphthalene mineralization time courses by *P. putida* strain 17484 in clay-free controls (□) and in 50% HDTMA-modified smectite slurries containing 2.5 (▲), 7.5 (o), 10 (△), or 25 (■) mg of clay ml^{-1}. 50% HDTMA-smectite was equilibrated with 100 ng ml^{-1} of naphthalene for 24 h prior to inoculation with strain 17484. Mineralization curves at 1 or 5 mg clay ml^{-1} were deleted for clarity. Initial rates (b) and extents (c) of naphthalene mineralization by strain 17484 in 50% HDTMA-smectite slurries (▲) and in a clay-free control (□) as a function of the equilibirum aqueous phase naphthalene concentration (lower x axis) as obtained by fitting the data in panel a to the coupled degradation-desorption model by non-linear regression.

Figure 2. a: Naphthalene mineralization time courses by *Alcaligenes* sp. strain NP-Alk in clay-free controls (□) and in 50% HDTMA-modified smectite slurries containing 1 (▲), 5 (o), 10 (△) or 15 (■) mg of clay ml^{-1}. 50% HDTMA-smectite was equilibrated with 60 ng ml^{-1} of naphthalene for 24 h prior to inoculation with strain NP-Alk. The mineralization curve at 7.5 mg ml^{-1} was deleted for clarity. Initial rates (b) and extents (c) of naphthalene mineralization by strain NP-Alk in 50% HDTMA-smectite slurries (▲) and in a clay-free control (□) as a function of the equilibrium aqueous phase naphthalene

concentration (lower x axis) as obtained by fitting the data in panel a to the coupled degradation-desorption model by non-linear regression.

Figure 3. Naphthalene mineralization time courses by *Alcaligenes* sp. strain NP-Alk in clay-free controls (□) and in slurries containing the < 0.25-mm (a) or the 0.25 - 1 -mm diameter (b) 50% HDTMA-modified smectite aggregates added at 1 (▲), 10 (○) or 35 (△) mg of clay ml^{-1}. 50% HDTMA-smectite was equilibrated with 60 ng ml^{-1} of naphthalene for 24 h prior to inoculation with strain NP-Alk.

Figure 4. Influence of sorption on the initial rates (a) and extents (b) of naphthalene mineralization as a function of the equilibrium aqueous phase naphthalene concentration in experiments with small (< 0.25-mm diameter, ○) and large (0.25 to 1-mm diameter, ▲) 50% HDTMA-smectite aggregates and in a clay-free control (□) inoculated with strain NP-Alk. Values were obtained by fitting the data in Figure 3 to the coupled degradation-desorption model by non-linear regression analysis.

Table 1. Properties of 50% HDTMA-smectite preparations.

Sample	% OC[a]	K_P[b]
Freeze-dried	8.43(0.49)[c]	185.67(10.68)
Oven dried		
<0.25 mm fraction	8.11(0.18)	221.31(1.83)
0.25 to 1 mm fraction	8.11(0.18)	208.57(22.89)

[a] Percent organic carbon (dry weight). [b] Naphthalene partition coefficient (L/Kg). [c] Values in parentheses represent one standard deviation.

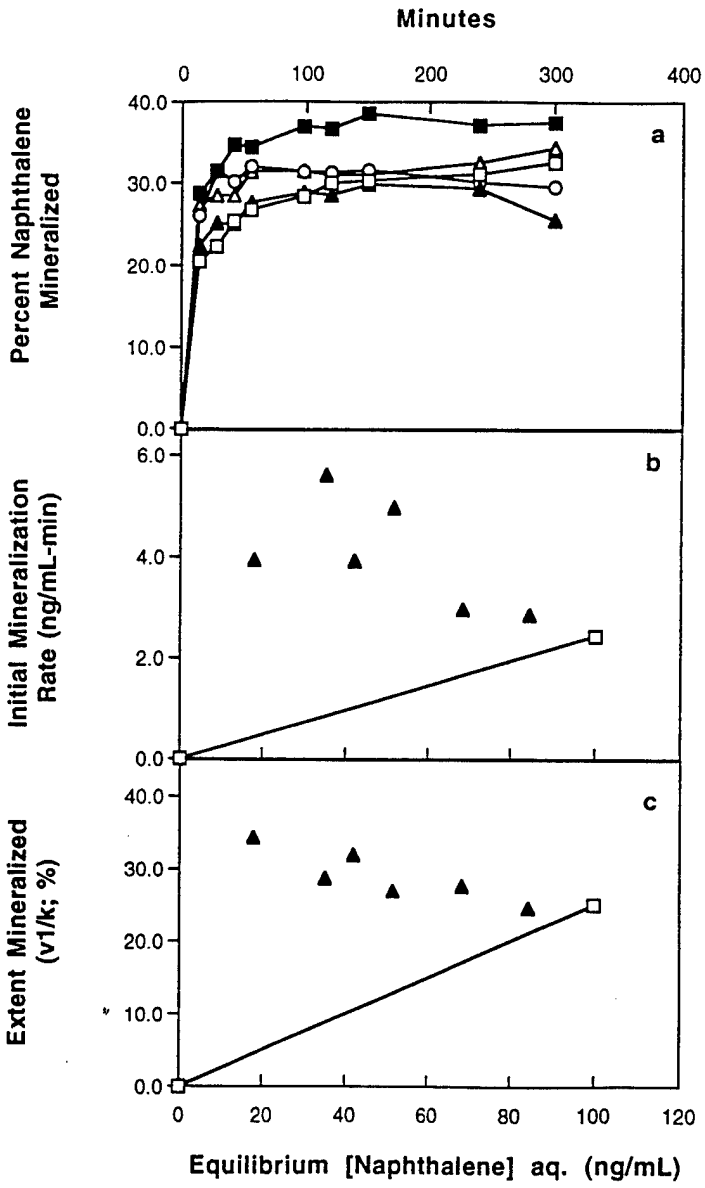
Minutes
0
100
200
300
400
Percent Naphthalene Mineralized
40.0
30.0
20.0
10.0
0.0
a
Initial Mineralization Rate (ng/mL-min)
6.0
4.0
2.0
0.0
b
Extent Mineralized (v1/k; %)
40.0
30.0
20.0
10.0
0.0
c
0
20
40
60
80
100
120
Equilibrium [Naphthalene] aq. (ng/mL)

FIGURE 1

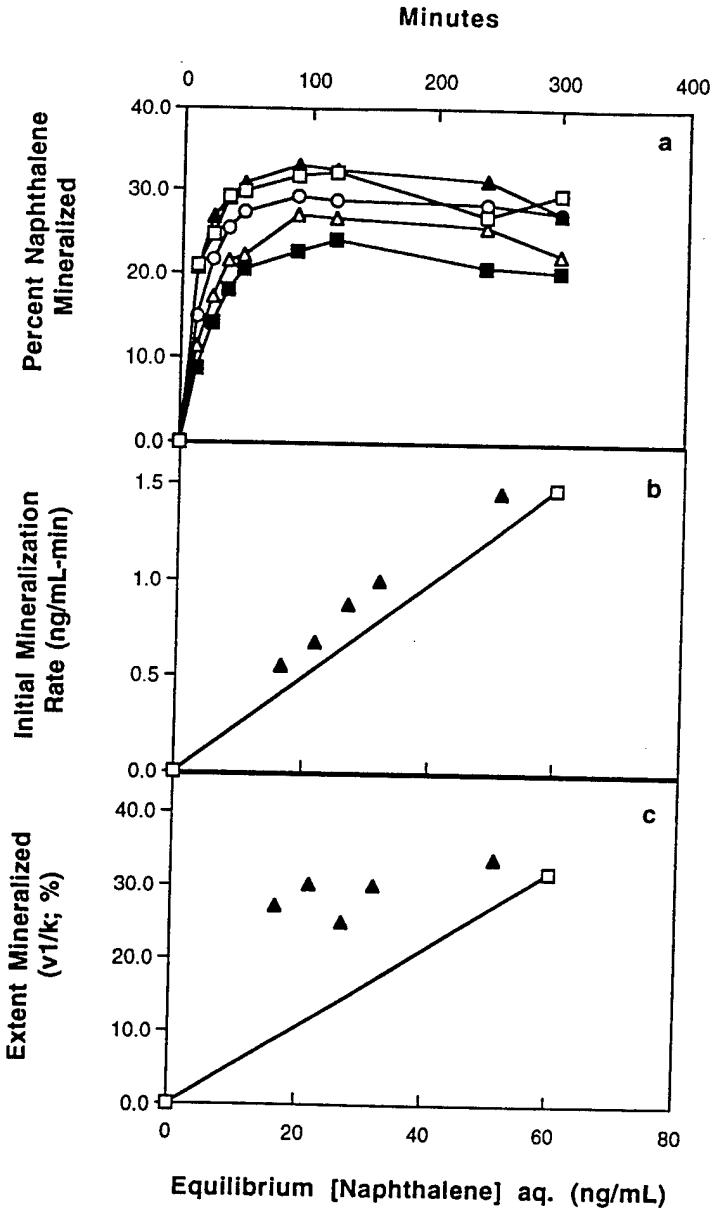
Minutes
0
100
200
300
400
Percent Naphthalene Mineralized
40.0
30.0
20.0
10.0
0.0
a
Initial Mineralization Rate (ng/mL-min)
1.5
1.0
0.5
0.0
b
Extent Mineralized (v1/k; %)
40.0
30.0
20.0
10.0
0.0
c
0
20
40
60
80
Equilibrium [Naphthalene] aq. (ng/mL)

FIGURE 2

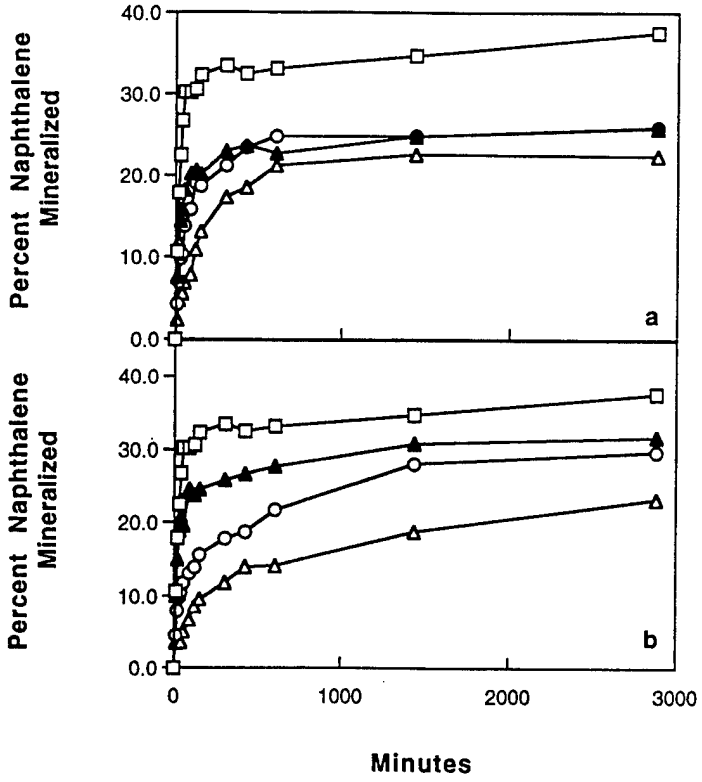
Percent Naphthalene Mineralized
40.0
30.0
20.0
10.0
0.0
a
Percent Naphthalene Mineralized
40.0
30.0
20.0
10.0
0.0
b
0
1000
2000
3000
Minutes

FIGURE 3

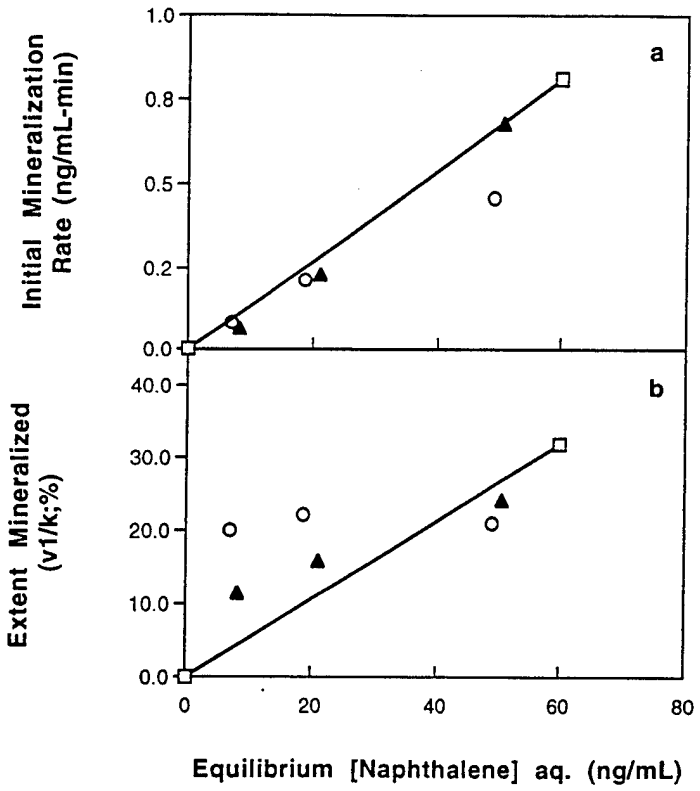
Initial Mineralization Rate (ng/mL-min)
1.0
0.8
0.5
0.2
0.0
a
Extent Mineralized (v1/k;%)
40.0
30.0
20.0
10.0
0.0
b
0
20
40
60
80
Equilibrium [Naphthalene] aq. (ng/mL)

FIGURE 4